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Two exquisite hemipteran galls of India with notes on the physiology of gall induction by Sternorrhyncha[#]

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ABSTRACT: The Indian subcontinent is rich with gall-inducing insects. The varieties of galls they induce offer bountiful opportunities to explain the dynamics of insect-plant interactions. Close to 90% of gall-inducing insects across the world are known to be specific to certain plants and such specialist behaviour offers them for use as ideal models to explain and characterize insect-plant relationships, which bear long-term advantages in managing insects that live and feed on economically important plants. In such a context, I illustrate in this paper, the intimacy of relationships between two gall-inducing Hemiptera (*Apsylla cistellata* tied to *Mangifera indica* and *Mangalorea hopeae* tied to *Hopea ponga*), which are native to the Indian subcontinent. In this article I emphasize that studying the biology of gall-inducing insects unequivocally demands a clear understanding of the stress and reparative physiology of the plant as well, further to that of the feeding biology of the inducing insect. Since all known gall-inducing insects (Hymenoptera excepted) induce galls by feeding action, I have explained the vitality of knowing about mouth parts, salivary secretions, and the mechanisms that arise in plants consequent to insect feeding with regard to the Hemiptera. My plea is that with the vast variety of various gall-inducing insects, we in India have a large canvas to paint the details of the physiology and metabolomics involved in insect-plant interactions clearly, because these insects are highly specialized in selecting their hosts, and also because these insects live embedded within plant tissues for certain period of time. In an ecological context, these insects are more easily amenable to monitor in field contexts than other free-living insects.

KEY WORDS: Peninsular India, Indo-Gangetic Plains, *Mangalorea hopeae, Apsylla cistellata*, *Contarinia manii*, dynamics of interactions

INTRODUCTION

Insect-induced galls have been recognized in India for long. For example, the medicinal relevance of the pouch galls that arise on the leaves of *Terminalia chebula* (Combretaceae) is mentioned in *Amarasimha*'s *Amarakôsã* of the 4th Century AD (Peyer, n.d). We know today that these galls are induced by *Dixothrips onerosus* (Thysanoptera: Phlaeothripidae) (Ananthakrishnan and Raman, 1989; Raman, 2013). Mani's *Cecidotheca Indica* (1959) served as a useful primer for Indian galls; revised editions of this monograph appeared as *Plant Galls of India* in 1973 and 2000.

In 2007(a), I wrote highlighting many of the ignored dimensions of this branch of entomology, intending that it would stimulate the study of the curious

* Author for correspondence # Invited Article

biologies of these specialist insects. On various scores, the gall-inducing insects of India are unique: a majority of the peninsular-Indian gall-inducing insect elements are endemic to this region, whereas a majority of the northern-Indian gall-inducing insect elements are not, mainly because of the interconnectedness of the Indian plate with the European plate. The restriction of the gall-inducing Cynipoidea (Hymenoptera) and Aphidoidea (Hemiptera) to the foothills and slopes of the Himalaya and the near cent-percent absence of gall-inducing Cynipoidea and Aphidoidea in Peninsular India (Raman, 2007b) reiterate the above. Nevertheless, given the long time periods over which the plants and animals of the Indian subcontinent have been evolving, galls - the expressed phenotypic expressions of tight interactions between two unrelated genomes - present an astonishing variety, concurrently raising umpteen scientific questions (see Raman 2007a, 2009a). Mani (2000) reports nearly 2000 types of galls with a majority of them displaying amazing morphologies. One extraordinary example will be the cylinder-piston gall induced on the opposite leaflets of *Acacia ferruginea* (Leguminosae, http://www.theplantlist. org/tpl1.1/record/ild-31791) from the vicinity of Coimbatore (Rohfritsch, 1971) (Fig. 1), which stand unmatched in the biological world. Keith Harris described the inducing Cecidomyiidae of these galls as *Contarinia manii* (Diptera) in 2010, whereas Mani when first found it in Walayar (Palghat gap, 10°232 N, 76°522 E) placed the insect under *Lobopteromyia* (Mani, 1953).

Unlike the bacterium-, fungus-, and nematode induced plant abnormalities, which I prefer to designate as 'tumours', those induced by insects (used heré to include the Acarina as well), usually presenting impressively symmetrical shapes, I prefer to call 'galls' (Raman, 2003, 2007a, 2009a). The tumours are amorphous, whereas galls are of definite, usually symmetrical, shapes.

In this article, I will be dealing with the biologies of and the sea-urchin like galls on *Hopea ponga* (Dipterocarpaceae) induced by *Mangalorea hopeae* (Hemiptera: Coccoidea: Beesoniidae) (Fig. 2) and the fir-cone like galls on *Mangifera indica* (Anacardiaceae) induced by *Apsylla cistellata* (Hemiptera: Psylloidea: Aphalaridae) (Figs. 3, 4). One reason for the choice of these examples is that both galls are induced on the axillary vegetative shoot buds by two Hemiptera. The *M. hopeae* populations occur restricted to the western coastal plains (Peninsular India, the Malabar Coast, Konkan Coast), whereas *A. cistellata* populations to the wider Gangetic Plains (27°152 N; 80°302 E). While consolidating known information of these two galls, I will speculate some details, further to offering a few general remarks on the gall flora and the inducing fauna of India. The speculation, I am confident, would encourage the present generation of Indian entomologists, especially those interested in exploring the ecology and physiology of insect–plant interactions, in proving me either right or wrong.

HEMIPTERAN GALLS

MANGALOREA HOPEAE AND GALLS ON *HOPEA PONGA*

Mangalorea hopeae belongs to the Beesoniidae (Coccoidea) (Raman and Takagi, 1992; Saleem and Nasser, 2015). Presently we know of *Beesonia* (four species), *Gallacoccus* (five species), and *Mangalorea*, *Echinogalla*, and *Danumococcus* (one species each). Except *Beesonia napiformis* and *B. brevipes* living on different Fagaceae in warm temperate eastern Asia, the remainder live on various species of the Dipterocarpaceae in warm, humid southern and south-eastern Asia (Takagi, 2007). A Neotropical taxon *Limacoccus* living on species of Arecaceae is currently listed under the Beesoniidae (Limacocciini) (Foldi, 1995), which appears odd. The curiosity is that the Fagaceae-infesting warm-temperate eastern Asian species of the Beesoniidae do not induce galls, whereas the known Dipterocarpaceae-infesting subtropical-tropical taxa induce galls (Takagi, 1987). Presently, the relationships within the Beesoniidae-those on Fagaceae and those on Dipterocarpaceae-remain unexplained (Takagi, 2007).

The earliest trigger to establishing the Beesoniidae, a unique family of the Coccoidea, was from India. Edward Ernest Green (Williams, 1999), a tea planter

in Ceylon (Sri Lanka) and an amateur mycologist-entomologist, described *Beesonia dipterocarpi*, which induces chrysanthemum flower-like galls on the vegetative shoot buds of *Dipterocarpus tuberculatus* in Burma, after he retired to UK (Beeson, 1941, pp. 743-744). The *B. dipterocarpi* specimens were sent to Green, from the Entomologist's office, Forest Research Institute (Dehra Dun) in 1926. Green refers to this 'new' insect as 'remarkable' and names it after Cyril Frederick Cherrington Beeson. Green (1928) offers emendations to his 1926 description and provides supplementary notes. Green, in 1926, did not assign this taxon to any subfamily then known (MacGillivray, 1921). He suspected that it could be a member of Tachardiinae; and at the same time, he also indicated that the adult males resemble those of *Conchaspis* (Conchaspinae) (Raman and Singh, 2014).

The galls of *Hopea ponga,* presenting similar to sea urchins, occur generally in leaf axils and rarely at the shoot terminals. Mature galls are dark green and spherical, endowed with numerous stiff and sharp structures (appendages, spines). With maturation, galls turn from pale to dark green, then to brownish green, and finally to grey, losing simultaneously their spherical shape and developing cracks. Usually only one gall occurs at an axil, although occasionally more occur. The following details are paraphrased from Raman and Takagi (1992).

Soon after the monsoon rains, the neonate female nymphal instars of *M. hopeae* invade the axillary angles of vegetative buds, exploiting the naturally occurring space due to extra-axillary position of the vegetative axillary bud. Once settled, the nymph feeds on the cortical parenchyma of the bud. The feeding stimulus restricts the bud from growing into a vegetative branch; instead, it develops into a gall, resulting in a structure that includes an eccentrically grown 'columella' that arches over the inducing nymph. Vascular traces ramify through the columella. Subsequent growth of the columella takes place essentially due to division of cells of the central cortex of the columella. Simultaneously with the arching growth of the bud meristem, some of the epidermal cells differentiate into multicellular, vascularized spiny structures. The stimulus provided by the feeding activity of the growing female nymph (the gall inducer) that occupies the space in the leaf axils of *Hopea* activates the epidermal cells to become multicellular, spiny structures. These structures on mature galls have lignified walls and polyphenolic inclusions.

In old galls, the columella is more striking than that of the spiny structures. With ageing, the parenchyma cells of the columella become lignified. Rupture of vascular strands disrupts water and nutrient supply to the gall. Lignified parenchyma cells separate from one another due to dissolution of middle lamella and develop large intercellular spaces. Cells bordering the gall stretch horizontally pulling the spine-like appendages on the lateral axis. Such lateral movement of appendages facilitates the escape of adults (to occur) from the gall.

APSYLLA CISTELLATA AND GALLS ON *MANGIFERA INDICA*

Galls of *Apsylla cistellata*, resembling the cones of Coniferae (now referred as Pinophyta), arise at the leaf axils of *Mangifera indica* through the modification of axillary vegetative shoot buds. Usually one gall arises at one leaf axil, although several may arise at the ends of branches. *Apsylla cistellata* is presently placed under Rhinocolinae, Aphalaridae of the Psylloidea (Burckhardt and Ouvrard, 2012). George Buckton described this taxon as *Psylla cistellata* in 1896 based on specimens sent to him from Dehra Dun. While describing *P. cistellata*, Buckton remarks that this taxon appears so 'curious' that a change of its generic name and status may be necessary. David Crawford, then at Hawaii, parked this taxon under a new name *Apsylla* in 1912. Mathur (1975) treated *A. cistellata* under Pauropsyllinae (Psyllidae). White and Hodkinson (1985) treated *A. cistellata* under the Calophyidae, with Psylloidea being recognized as a superfamily. A comprehensive list of previous papers dealing with cursory biological investigations of *A. cistellata* is available in Raman *et al*. (2009a). Later papers on *A. cistellata* by Shivankar and Rao (2010) and Jha *et al*. (2013)

essentially deal with the economic damage caused by these insects to *M. indica* and how *A. cistellata* can be managed with chemical applications. Almost all of these papers refer to *A. cistellata* as a 'serious pest' of *M. indica*, but none clarifies to what extent *A. cistellata* either affects economic productivity or damages *M. indica*.

In spite of scores of papers published on the management of *A. cistellata*, including the lengthy monograph by Gajendra Singh (Singh, 2003), a clear knowledge of the bionomics of this curious insect is still deficient. I summarize the details available in various papers of Gajendra Singh here: Gravid females insert 75-150 eggs along the midribs of newly flushed leaves in March-April in two parallel rows. The newly deposited, oval eggs are whitish and translucent with its tip partly exposed (Singh and Misra, 1978). The eggs hatch in either mid-September or early October, approximately 200 days after oviposition. Nymphal phase includes five instars and the development into adults takes *c*. 140 days. Gravid females never oviposit on the leaves of seedlings, but only on the tender leaves of older plants that are about to flower and bear fruits (Singh, 2003). Feeding action of the first-nymphal instar initiates the gall. The neonate nymphal instars remain partly within egg shells and feed on the same leaf where the adult female oviposited (Singh *et al.*, 1975). The feeding effect of multiple neonate nymphs results in the modification of 'adjacently' occurring vegetative shoot buds into galls in about 30 days. Singh (2000) indicates that an increase in endogenous auxin levels and a decrease in total phenols and levels of tyrosine and tryptophan occurs in the shoot buds of *M. indica* that grow into galls. Singh (2003) further indicates a correlation between age of flowering and gall incidence.

The emerging message is that the neonate nymphal instars of *A. cistellata* feed on *M. indica* leaves, particularly on those, which harbour eggs. Feeding action stimulates gall development, not at the same site, but at a site farther away, *viz.*, the vegetative axillary shoot bud by translocating a chemical 'stimulus'.

REMARKS

By talking about two extremely fascinating galls of India, I aim to instil curiosity and interest in Indian entomologists and ecologists who deal with insect-plant interactions, so as to explore these dynamic systems further. I also attempt to compare these systems with a few explained galls induced by other Sternorrhyncha and a few Auchenorrhyncha. At this juncture, it would be pertinent to recognize that the claims of gall induction by the Auchenorrhyncha are of recent times (Matsukura *et al.*, 2009, 2010). They are questionable in terms of the concept of a gall, but are indicated as galls by their authors. For those interested in the study of galls, reading Meyer (1987) would be most fundamental, which explains the basic concepts in gall-inducing insect–plant interactions fascinatingly, with hundreds of examples drawn from all over the world, although several other books on the biology and ecology of gall-inducing insects have appeared later (*e.g.*, Shorthouse and Rohfritsch, 1992; Raman *et al.*, 2005a).

APSYLLA CISTELLATA AND *MANGALOREA HOPEAE*

Gall-induction behaviour of *A. cistellata* stands strikingly different from what could be perceived as the basic pattern among the other better known and more diverse gall-inducing Psylloidea - the Triozidae (Burckhardt, 2005). Before I proceed to make any comparisons, it would be pertinent to recall the biology of feeding by the Adelgidae (Hemiptera: Aphidoidea) here. Adelgidae bear very long stylets; much longer than their total body lengths and longer than the other Aphidoidea do (Rohfritsch, 1990). For example, the stylet bundle lengths of nymphal instars of *Adelges piceae* (Adelgidae) are nearly five times longer than their body lengths. The staggering length of stylets in the Adelgidae is adapted not just for feeding, but also to anchor them on the shoots they feed on (Young *et al.*, 1995). Similar details are available in Rohfritsch (1990) referring to *A. laricis* and *A. abietes* that induce shoot bud galls on *Picea excelsa* in Europe. In

Adelges cooleyi, which induces galls on the vegetative shoot buds of *Picea glauca* × *P. engelmannii* hybrid in North America, Sopow *et al.* (2003) indicate that a dose-dependent chemical stimulus either moves actively or is moved passively over long distances from the point where the gall-founding female occurs. The overall gall-inducing behaviour of the Indian taxon *A. cistellata* appears highly similar to what is known in the European and North-American Adelgidae, which leaves us baffled with several questions: Is the behaviour known in the Adelgidae, an aphidoid, reappears in *A. cistellata*, a psylloid? Is the stylet of *A. cistellata* immensely long, which is inserted at one point (*viz.*, the leaf on which the neonates emerge) and their tips reach a distant point (*viz.*, the vegetative bud at the leaf axil), similar to what has been shown in *A. piceae*, *A. laricis*, and *A. abietes*? On the contrary, the stylet tip does not reach the vegetative buds, but as shown in *A. cooleyi* the salivary secretions (the stimulus) are transmitted to a distant point thus triggering gall development at another site? In spite of an apparent similarity, in the *A. cistellata*-induced bud galls on *M. indica*, the first-instar nymphal instars of *A. cistellata* are the gall initiators, whereas in the bud galls induced by various Adelgidae, adult females are the gall initiators (= the *fundatrigeniae*). Notwithstanding the above similarity in insect behaviour by stimulating galls at sites far away from where actually the initiating insect stages reside, the question raised by Prasad (1957), whether *A. cistellata* plays a vectorial role in transmitting a virus, which possibly stimulates gall development, merits investigation given that many Psylloidea are established vectors of plant pathogens.

Apsylla cistellata populations remain restricted to the Indo-Gangetic Plains and lower valleys of the Himalaya; however, Kandasamy (1986) has reported its incidence in the Shevaroy Hills (11°46'N; 78°12'E; 700–1200 m a.s.l.) in humid, tropical peninsular India, which has not been verified subsequently. Although *M. indica* grow extensively in several warm parts of the world, *A. cistellata* is not known to occur in any geographical area other than the northern plains of the Indian subcontinent including parts of Pakistan, Bangladesh, and Nepal. A possible reason for the localized incidence of *A. cistellata* is the annual rainfall of more than 1100 mm and a difference of more than 30°C between the highest maximum and lowest minimum temperatures (Singh 2003).

Two principal life stages of *Mangalorea hopeae* participate in *Hopea* gall system: (i) one female first-nymphal instar initiates the gall on a vegetative shoot bud exploiting the extra-axillary space; (ii) several male nymphal instars, emerging from that female after its maturation and mating, move and occupy spaces between the sharp spiny structures. The males occurring between such structures alter gall physiology by their feeding, particularly in ageing galls. Because of their number, they utilize nutrients more vigorously than what an ageing gall can mobilize, which accelerates drying of galls. The occupation of the maternal gall by several male nymphal instars is not unique to *M. hopeae*. *Cystococcus* (Coccoidea: Eriococcidae) shows this behaviour that the male offspring complete their development within the maternal gall on *Eucalyptus,* feeding on a layer of nutritive tissue lining the gall cavity (Gullan and Cockburn, 1986). Gullan and Cockburn (1986) also speak of dispersal of the second and subsequent generations of nymphal instars by the first generation of winged males, which explains dispersal of apterous female nymphal instars. Does a similar phoretic phenomenon possibly occur in the biology of *M. hopeae*? This question needs to be answered.

The terminal regions of generative buds are not damaged during gall induction, since the gall-founding female *M. hopeae* feeds only along the sides. The cecidogenetic gradient activated by the feeding stimulus spreads to apical segments of the gall, promoting an expansive growth of the host bud establishing the gall columella. With the disturbance of normal morphogenetic controls, the transformed apex, instead of initiating leaf primordia (and later, the branch), undergoes intense parenchymatization and negotiates a curvature, providing cover to the gall-initiating female simultaneously. Cecidogenetic stimulus also triggers a rare developmental course transforming columella's surface cells into multicellular, vascularized structures. During their

initial phase of growth, the terminal, lance-like parts of the spiny structures exhibit a more active growth than the lower stalk regions of these appendages. The lance-like parts of adjacently occurring spiny structures occur so closely that they physically protect inhabiting nymphal instars. Lower stalk region of each spiny structure elongates more intensely by stretching than by cell division and the entire appendage complex is strengthened by the vascular network of the columella. With maturation, the columella cells stretch in the horizontal axis due to desiccation resulting in the separation of appendages, thereby facilitating the escape of adult males.

GALL - INDUCING BEHAVIOUR OF *APSYLLA CISTELLATA* AND *MANGALOREA HOPEAE VIS-À-VIS* OTHER HEMIPTERA

A few common patterns can be discerned in the gall-inducing behaviour in the Sternorrhyncha: (i) gall initiation is usually by the feeding action of a single, adult female; (ii) the gall-founding females disperse over short distances seeking juvenile plant organs, such as tender shoot terminals and differentiating leaves (Raman 2012a). The gall-inducing Adelgidae and Beesoniidae differ from this pattern in such a way that, neonate, female nymphal instars initiate galls. Among the gall-inducing Triozidae (Psylloidea) the first-instar nymphs initiate galls by settling on stomatal apertures and feeding through the stomatal apertures. However, in the Triozidae, whether the initiating nymphal instar is a female or a male is uncertain presently, although the chances of a male inducing a gall are highly unlikely. Among the Psylloidea, the gall-inducing behaviour of *A. cistellata* appears markedly different compared with those of the gall-inducing Triozidae and Psyllidae (Psylloidea).

In the gall-inducing Triozidae, gravid females deposit their eggs at the same site where the galls would develop, and only the egg stalks remain buried in the plant tissue. In contrast, the eggs of *Apsylla cistellata* remain 'partly buried' on the leaves of *M. indica* and the nymphal instars that emerge from those eggs feed on the same leaf, but their feeding action triggers gall induction on the axillary vegetative buds, at least 10 cm away. Samui and Jha (2009) provide a slightly more detailed description of *A. cistellata*'s oviposition behaviour: (i) the eggs are laid singly in slits cut using the ovipositor, those eggs remain embedded in midrib tissues along the under sides of new leaves; (ii) eggs are inserted alternatively by puncturing the tissue along both sides of the dorsal face of the midrib; (iii) the intensity of egg laying depends on the availability of new flush of tender leaves and the number of adults emerging, and (iv) if several females had only a few leaves for egg laying, then they lay eggs along both sides of lateral veins along the under sides of *M. indica* leaves. Burying eggs in host tissue, as evident in the behaviour of *A. cistellata*, therefore, emerges as a special, non-Triozidae trait in the Psylloidea.

Claims of gall induction by the Auchenorrhyncha need to be referred here. The earliest records of Auchenorrhyncha-induced 'galls' exist from the 1920s, referring to *Philaenus spumarius* (Cercopoidea: Aphrophoridae) on *Oenothera* (Onagraceae) and *Ceresa bubalus* (Cicadoidea: Membracidae) on *Medicago sativa* (Leguminosae) (Meyer, 1987: pages 92-93). In terms of general biology, more details are available for the Tingidae (Heteroptera: Cimicomorpha), which prefer to feed on the abaxial-leaf sides seeking humid microenvironments - a trait shared by many gall-inducing Sternorrhyncha. Nonetheless, among the supposed gall-inducing Tingidae (*e.g.*, *Copium* and *Paracopium*), their preference for flowers and capability to induce floral galls impress as specialized traits among gall-inducing Hemiptera (Schaefer, 2005), because floral galls induced by the Sternorrhyncha are not known. Gall-bearing *Teucrium polium* (Lamiaceae) (Sinai desert, Egypt; 29°30'N; 33°50'E) include leaves and floral axes reduced in overall size, although the petals in galled flowers were 'enlarged' (Zalat *et al*., 2000). The other key behaviour that distinguishes gall-inducing *Copium* from gall-inducing Sternorrhyncha is that they bury their eggs- nearly fully - in host tissues (Behr, 1952; Monod and Carayon, 1958). A differently structured internal reproductive system in *Copium* is implicated to be better adapted for such a specific behaviour (Schaefer, 2005).

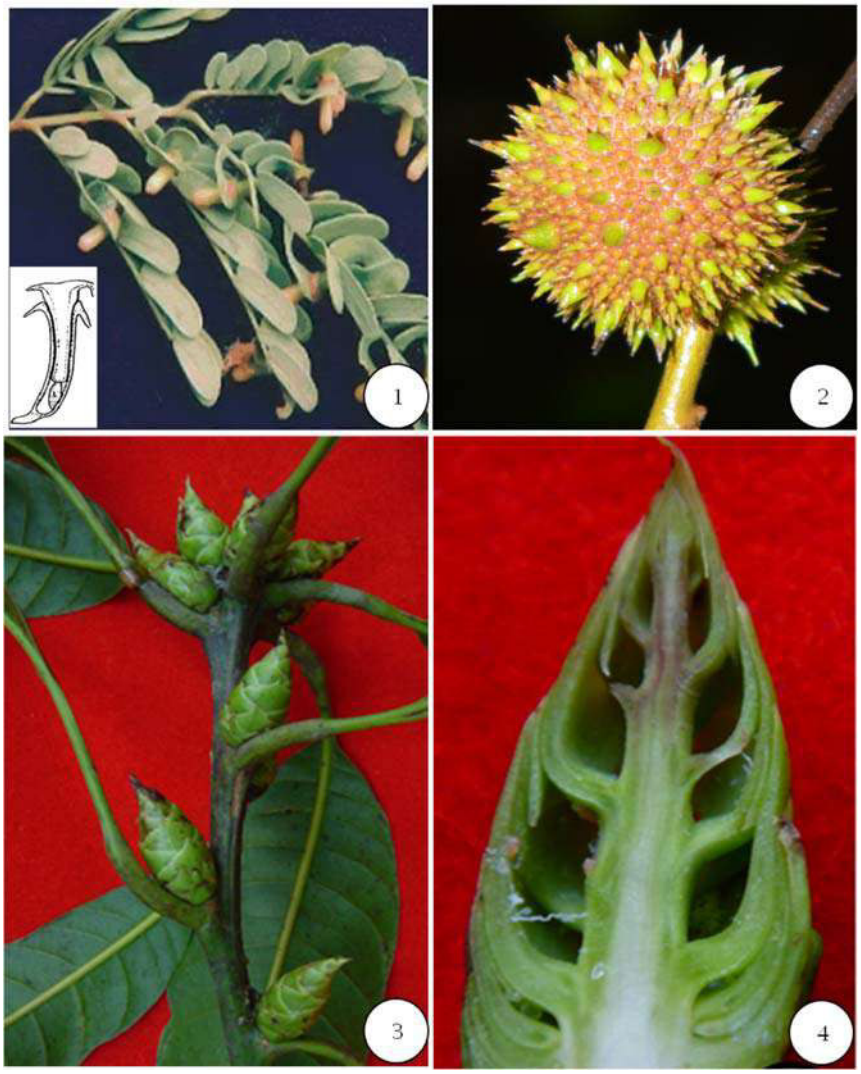

Fig.1. Cylinder-piston galls on *Acacia ferruginea* induced by *Contarinia manii* in southern India. Inset:: vertical longisectional drawing showing the position of the inducing larva (L).
Fig. 2. Sea-urchin shaped galls on the shoot buds of *Hopea ponga* induced by *Mangalorea hopeae* along the Malabar Coast. (Photo courtesy: M. Nasser, Calicut University, Calicut).
Fig. 3. Coniferae cone like galls on the shoot buds of *Mangifera indica* induced by *Apsylla cistellata* distributed along the Indo-Gangetic Plains.
Fig. 4. Vertical longisectional view of one gall showing nymphal instars and chambers.

Parallelism in the 'gall'-inducing behaviour in Auchenorrhyncha on the one hand and in the few gall-inducing Terebrantia (Thysanoptera) (*e.g.*, *Aneurothrips preisneri*, Thripidae, on *Cordia dichotoma*, Boraginaceae) on the other is more striking. *Scenergates viridis* (Hemiptera: Cicadellidae) are indicated to induce 'gall'-like structures by modifying the entire leaves of *Alhagi maurorum* (Leguminosae) (Ratikov and Appel 2012), which strikingly resemble the leaf-fold galls induced by *Gynaikothrips ficorum* (Thysanoptera: Phaleothripidae) on the leaves of *Ficus microcarpa* (Moraceae). Among the known instances of gall induction in the Cicadellidae (Mitjaev, 1968; Matsukura *et al.,* 2010), a common behaviour is that both the juveniles and adults induce galls, which are different from that known among gall-inducing Sternorrhyncha. Nymphal instars and adults of

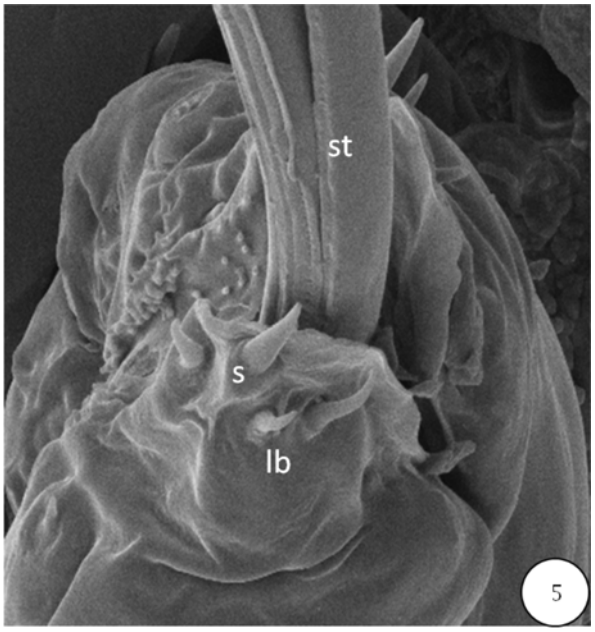

Fig. 5. Mouth parts of a gall-inducing species of *Glycaspis* (*Synglycaspis*) (Psylloidea: Aphalaridae). s – sensillum; lb – labium; st – stylet bundle
(Source: Sharma *et al*., 2015)

Cicadulina bipunctata (Hemiptera: Cicadellidae) induce galls not only at the locations they feed but also on distant leaves through dose-dependent stimulation (Matsukura *et al*., 2009); this behaviour is similar to the gall-inducing behaviour of *A. cistellata* and *A. cooleyi*. As of the present, I will summarize that gall-inducing behaviour is uniquely preserved predominantly among the Aphidoidea, Psylloidea, and Coccoidea and to an insignificant extent in the Aleyrododoidea (one biotype of *Bemisia tabaci* inducing colourful, parenchymatous galls on the leaves of *Achyranthes aspera* (Amaranthaceae)) in the Indian subcontinent. Sporadic papers refer to certain plant abnormalities due to sucking-feeding behaviour among various Auchenorrhyncha, similar to the papers that refer to gall induction by a species of the Chironomidae (Diptera) on the different aquatic plants (Raman, 2009b; Jäger-Zürn *et al.*, 2013). In such vague contexts, it is but critical that we progress ideas with extreme care.

MOUTH PARTS, FEEDING BIOLOGY, AND PHYSIOLOGY OF GALL INDUCTION IN STERNORRHYNCHA

Many recent papers explain the morphology of mouth parts of plant-feeding Hemiptera, mostly referring to the Aphidoidea, which we therefore need to use as a basic model. The mouth parts include the labrum, labium, and a sclerotized stylet bundle, which in turn, includes paired mandibular and maxillary stylets. This 'mouth-parts complex' is essentially tubular and devoid of either labial or maxillary palpi. The labral cone, usually endowed with sensilla, is attached proximally to the clypeus and occurs overarching the labial groove. The included stylets are pointed and are elaborately sculpted both at the tips and along the edges. The first maxillae are tightly adpressed to each other so that the oppositely lying grooves along their interfaces arrange in such a manner that they bear two superposed capillary tubes (Fig. 5). Through one, the feeding Hemiptera flushes its saliva and through the other, sucks plant sap. Endowed with a variety of sensilla, the distal tip of the labium guides the stylet into the host organ. The second maxilla fused into a labium constitutes the rostrum, with a groove in which the distal parts of the stylets slide. Each stylet is manipulated by two sets of retractor and protractor muscles. Muscles attached to the ceiling of the cibarium provide suction, which helps in either drawing or injection through the food and salivary canals that lie between the maxillary stylets. The two maxillary stylets interlock with each other along their full length, thus constituting a smooth hollow tube that bears an armature of denticles at the tips (Hori, 2000). The articulation on the opposite side of the stylet bears the salivary canal, which opens terminally between the denticles and the extreme end of the stylets. Although each maxillum is similar in shape and dimension, lengths of stylets change as the insect grows: for example, in the first nymphal instars of Psylloidea it is usually 300 - 600 mm long, whereas in adults of Psylloidea it is 1000 - 1400 mm. Because the stylet bundles become longer with each successive moult of nymphal instars, developing nymphs shift feeding sites from superficial to deeper-lying plant cells as they mature. For example, the gall-founding female

Adelgidae change their feeding sites several times during gall development (Rohfritsch and Anthony, 1992). During feeding, the labium does not pierce the plant tissue, but is positioned perpendicular to the surface so as to push the stylets into the plant. Although a majority of the Sternorrhyncha feed passively on phloem contents, several studies on gall-inducing Sternorrhyncha, especially on the nymphal instars, indicate them to be nonvascular tissue feeders (*e.g.*, parenchyma) (Raman, 1991; Rohfritsch and Anthony, 1992; Sharma *et al.*, 2014).

Hemiptera, specifically the Aphidoidea, produce two types of saliva. The first is dense and proteinaceous, which gels around the stylets forming stylet sheaths, isolating the plant tissues from the mouth parts, and preventing any possible adverse plant reactions (Felton and Eichenseer, 1999). On reaching the target feeding site, they secrete the second type of saliva - less dense, and therefore the watery saliva - which is injected directly into plant tissues. The watery saliva contains diverse digestive and lytic enzymes. The feeding action inflicts a 'subtle' wound, but the salivary proteins interact with Ca^{2+} of host-plant tissues (Will *et al*., 2007; Sharma *et al.*, 2014) preventing the possible wound-healing effort made by the plant. In general, wounding does not either induce or result in cell necrosis. Stylet penetration occurs by changes in the position of the head during feeding; the head is bent over the labium, which is attached to the plant surface, forcing the stylet bundle down the labial groove, and into the host tissue (Freeman *et al*., 2001). Stylet tracks (the proteinaceous sheaths) are left behind within host tissues by the gall-inducing Sternorrhyncha after the withdrawal of the stylets. These tracks accept colouring by cationic dyes (*e.g.,* methylene blue, bismark brown) and can be easily detected under a good-quality light microscope. In some species, the track is straight, as evident in *Eriosoma lanigerum* (Aphidoidea: Pemphigidae), whereas in others it could be meandering and branched, as evident in *Adelges abietes* (Aphidoidea: Adelgidae). Some sternorrhynchs extensively explore the plant surface before commencing feeding (Lewis and Walton, 1958), whereas others do not (*e.g. Daktulosphaeria vitifoliae*, Aphidoidea: Phylloxeridae; Raman *et al.*, 2009c). In a majority of instances, the stylet path travels intercellularly dissolving the middle lamella, principally made of pectic compounds (Rohfritsch, 1976, 1988). Pectinase activity in aphid saliva is known from the time of Jacques Auclair (1963).

Injection of saliva alters the hormonal balance in the host, leading to gall development. For a detailed commentary on the presently valid explanations of gall-induction mechanisms, please refer to Raman et al. (2005b). A few supplementary points are summarized here: Triacylglycerides containing (E,E,E)-octa-2,4,6-trienoic acid from the galls induced by *Colopha moriokaensis* (Aphididae: Pemphiginae) on *Zelkova serrata* leaves (Ulmaceae) are indicated to be responsible for cell hypertrophy (Otha *et al.,* 2000). Soluble proteins in the saliva of the nymphs of *Trioza jambolanae* have been implicated as a critical factor for gall development (Rajadurai *et al.*, 1990). In the saliva of *Trioza apicalis*, an undetermined amine has been shown, which is indicated as the stimulating chemical (Markkula *et al.*, 1976). Gall-inducing Sternorrhyncha vigorously take up oxygen from the gall tissue (several examples in Miles, 1999), along with a stimulation of auxin activity. Use of oxygen in the tissues under arthropod attack might be so great that the IAA-oxidase activity that regulates the concentration of IAA might be deprived of oxygen and therefore inhibited. Such a deprivation of oxygen (Florentine *et al.,* 2002) results in the concentration of IAA increasing disproportionately at feeding sites with a consequential hypertrophy of meristematic plant tissues. Although the specific agent in the hemipteran saliva that induces galls has not been determined, salivary oxidases should be playing a role in the disruption of IAA-oxidase pathway.

CONCLUSION

One key characteristic of gall-inducing insects is their specificity to particular host plants. One possibility is the absence of resistance-breaking genes in gall-inducing insects. Lack of such genes explains why these organisms have not radiated and diversified aggressively as many other insects

have. On the contrary, host-plant populations are restricting the gene flow between specific gall-inducing insect populations, through their secondary chemistry because, the host-plant mediated impediments on the breeding behaviours impact on the radiation of gall-inducing insects (Raman 2012b). What can be said in conclusion is that the gall-inducing insects of the Indian subcontinent, more especially the Cecidomyiidae (Diptera), show features of conservative diversification (Raman et al. 2009a), whereas we know either little or nothing of the gall-inducing Hemiptera. Nevertheless, whatever little has been documented so far, appear to be strongly plant mediated, as evident in the instance of *Trioza fletcheri minor* (Hemiptera: Triozidae), which induces galls on more than one species of *Terminalia* (Combretaceae) (Raman *et al*., 1997). Within the Hemiptera, gall-inducing habit appears to have evolved multiple times, most of species diversity restricted to within few groups of the Aphidoidea, Psylloidea, and Coccoidea. More critically, gall-inducing behaviour varies strikingly even within the Hemiptera pointing to their independent evolution over time.

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Report of dung beetles (Scarabaeidae: Scarabaeinae) attracted to unconventional resources, with the description of three new species

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ABSTRACT: Dung beetles found attracted to and feeding on resources other than animal excreta and vertebrate carcasses were collected from different parts of India. Out of the 13 species collected nine were from millipede, three from snail and one from fungus. Of these three species *Onthophagus jwalae, O. pithankithae* and *O. tharalithae* are new to science; the former two were found feeding on millipede carcasses while the latter on a dead snail. *O. rudis* Sharp was found feeding both on live and dead millipedes. © 2016 Association for Advancement of Entomology

KEYWORDS: Coprophagy, fungus, millipede, necrophagy, saprophagy, snail

INTRODUCTION

Though the true dung beetles generally feed and breed in vertebrate excreta, many can survive on vertebrate carcasses and hence are termed as copro-necrophagous. Among the species which are carrion feeders a few are obligatory, while a few are reported feeding on insects and millipede carcasses (Pereira and Martinez, 1956; Howden and Young, 1981; Janzen, 1983; Gill, 1991) and even on decaying vegetable substances (Arrow, 1931). The ancestral scarabaeines were either saprophagous or fungivorous (Philips, 2011) and the availability of greater quantity of mammalian dung after the divergence of mammals, promoted the evolution of coprophagy from saprophagy (Cambefort, 1991).

The shift from coprophagy to necrophagy in most tropical forests can be attributed to the absence of large herbivores and to relative scarcity of necrophagous insects which can be potential competitors for the dung beetles (Halffter and Matthews, 1966). Necrophagy helps to acquire the required nitrogen content to build up muscles and in the case of females to mature their eggs. The mobile adults opt for more nitrogen rich omnivore dung or carcass for their nutritional requirements while they provide their brood with more abundant, carbohydrate rich herbivore dung (Hanski and Cambefort, 1991; Halffter and Matthews, 1966). It has been reported that a few such necrophagous species have opportunistically turned to predation (Halffter and Matthews, 1966).

There are several records of dung beetles being attracted to millipede defensive secretions and feeding on their carcasses (Krell *et al.,* 1997; Kon

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et al., 1998; Krell, 1999; Brühl *et al.,* 2003; Schmitt *et al.,* 2004; and Krell, 2004). Such dung beetles are attracted only to the millipedes belonging to the orders Spirostreptida and Spirobolida which can be attributed to the chemical composition of their defensive secretions which contain quinone derivatives i.e. 2-methyl-3-methoxy-1, 4-benzoquinone and 2-methyl-1, 4-benzoquinone (Smolanoff *et al.*, 1975). *Onthophagus latigibber* d'Orbigny, *O. bartosi* Balthasar and *O. mankonoensis* Balthasar were found to be attracted to fresh specimens of dead millipedes even before the defensive secretions had evaporated (Krell *et al.,* 1997). The few species which use the defensive secretions of diplopods as olfactory attractant for resource tracing have a major advantage by being the first to utilize this resource. In millipedes, the defensive secretions also act as pheromones for intraspecific communication. They use the defensive secretion as sexual signals during copulation (Haacker, 1974). There were reports of necrophagous scarab beetles, *Onthophagus rudis* Sharp and *O. penicillatus* Olsoufieff belonging to the sub- genus *Parascatonomus*, being attracted to a diplopod copulating pair which were soaked in their stinky secretion. It was observed that although the dung beetles just hid near the diplopod pairs, they did not try to attack or prey on the live diplopods (Kon *et al.,* 1998; Masumoto, 2001). The majority of the dung beetle species found to be feeding on the millipedes belong to the genus *Onthophagus* Latreille (Halffter and Matthews, 1966; Cambefort, 1983). In South Africa the genus *Sceliages* Westwood makes brood balls using millipede carcasses (Bernon, 1981). Further there have been reports of *Deltochilum kolbei* Paulian, *D. valgum acropyge* Bates and species of *Canthon* Hoffmannsegg predating and feeding on live millipedes (Halffter and Matthews, 1966; Cano, 1998; Villalobos, 1998; Larsen *et al.,* 2009).

Though there have been several previous records of dung beetles feeding on millipede carcasses and being attracted to their defensive secretions, there has been no report of dung beetles feeding on live millipedes from the India Subcontinent. In this paper, we report the observational record of thirteen dung beetle species being attracted to unconventional resources like millipedes, snails and fungus, out of which three species are new to science. The dung beetles discussed here were collected during various field trips to different parts of India.

MATERIALS AND METHODS

The beetles which were found feeding on resources other than animal excreta and vertebrate carcasses were picked up randomly during the various field visits and were preserved in 95% alcohol, brought to the lab, pinned, dried, identified, labelled and stored in the insect collection at ATREE Insect Museum, Bangalore (AIM-B).

The aedeagus was gently pulled out using forceps and needle through the opening of the pygidium after it was relaxed using a mixture of benzene, acetone and alcohol in the ratio 10:45:45. It was then point-mounted, measured and described. Both the insect and aedeagus was measured using micrometer fixed to a Mikrotek Binocular microscope. Species identification was carried out using the keys in Arrow (1931) and Balthasar (1963). Original literatures were referred for those species which were described later. Those which could not be keyed out to any known species were compared with the nearest species, designated as new and described.

Details of abbreviations for measurements are as follows: Total body length (TL) = distance from apex of clypeus to tip of pygidium; body width (BW) = maximal distance between lateral elytral margins; pronotal length (PL) = medial length of pronotum; pronotal width (PW) = maximal width of pronotum; elytral length (EL) = elytral sutural length; head length (HL) = medial length of head; head width (HW) = maximal distance between the sides of head.

Aedeagus measurements: Length of phallobase (LP) = distance from base of phallobase to the point of articulation with parameres; breadth of phallobase (BP) = broadest width of the phallobase; length of parameres (Lp) = distance from the point of articulation with phallobase to the tip; breadth of parameres base (BpB) = width of parameres at

the base; breadth of parameres tip (BpT) = width of parameres at the tip.

Images were taken using Canon 70D SLR camera mounted with Canon MP- E 65 mm macro lens and twin-lite flash. Combine ZM stacking software was used to stack the series of images taken at different focal points and the scale for the images were provided using Image J Software.

RESULTS

Out of the 13 species of Scarabaeine dung beetles recorded, nine species, *Onthophagus arboreus* Arrow, *O. coeruleicollis* Arrow, *O. malabarensis* Boucomont, *O. pygmaeus* (Schaller), *O.* (*Parascatonomus*) *rudis* Sharp, *O. tritinctus* Boucomont, *O. vultur* Arrow and two new species, *O. jwalae* and *O. pithankithae* were found feeding on millipede.Two species, *O. furcicollis* Arrow, along with another new species, named *O. tharalithae* were found feeding on dead giant African snail (*Achatina fulica* Bowdich), while another species, *O. igneus* was found feeding on dead unidentified snails. A single specimen of *Delopleurus parvus* (Sharp) which was considered to be rare, as they were not common in collections using dung baits was found under a puffball fungus. *O.* (*Parascatonomus*) *rudis* is seen attracted to the defensive secretion of an injured live millipede (Spirostreptida) and were also collected on dead millipede. One individual of this species was noticed as trying to gain entry into a millipede which was running about in distress and another of the same species was found inside the body of that millipede, which might have entered through its damaged posterior segments.

The following are the diagnostic characters to distinguish these species and descriptions of the three new species.

***Delopleurus parvus* (Sharp)**
(Plate 1, Image a)

Coptorrhina parva Sharp, 1875: 47 (original description),
Arrow, 1931: 410, 411 (key & description);
Balthasar, 1963: 278 (monograph);
Frovlov, 2014 (revision);
Delopleurus cardoni Paulian 1934 (synonym).

Diagnosis: Black, shining, highly convex; antennae and mouth- organs red, antennal club yellow; clypeus quadridentate; head densely rugosely punctured; basal margin of the pronotum with series of minute notches, median groove extending to quarter; elytra finely striate, striae with strong widely spaced punctures, deep angular sinuation on outer margin little behind the shoulder; pygidium reflexed ventrally, strongly transverse, its surface smooth, hollowed except for an abruptly raised margin; metasternum smooth, unpunctured, sides of metasternum fairly closely and shallowly pitted.

Measurement: TL = 5 - 6 mm, BW = 3 - 4 mm, PL = 2.13 mm, PW = 3.48 mm, EL = 3.05 mm, HL= 1.42 mm, HW = 2.13 mm.

Material examined: 1ex. (♂, AIM-B_ Co/ Sc1000133), "India, Karnataka, Regional Reference Standards Laboratory Campus, Jakkur, Bangalore; 5. VIII. 2012, Collected by Seena Narayanan Karimbumkara (SNK)".

Distribution: India: Odisha, Tamil Nadu, Kerala, Karnataka.

Type: Muse´um National d'Histoire Naturelle (MNHN), Paris, France (M. Rene Oberthür's collection).

Remarks: This species was found under a pufball fungus (Basidiomycota). Eventhough the genus *Delopleurus* is classified withdung beetles, they have been always been reported to be associated with basidiomycetes (Frolov, 2014).

***Onthophagus arboreus* Arrow**
(Plate 1, Image b)

Arrow, 1931: 222, 225 (original description);
Balthasar, 1963: 276 (monograph).

Diagnosis: Dark metallic green or coppery, elytra black, antennae bright orange, upper surface with inconspicuous pale setae; elongate- oval, highly convex, deeply waisted; head short, broad, flat, sides

bluntly angulate before the eyes; clypeus transversely rugose, front margin rounded; pronotum with strong longitudinal median impression posteriorly, front angles very blunt; elytra shallowly striate, intervals slightly convex, not very shining, finely sparingly asperate- punctate; pygidium shining, not closely nor very finely punctured; metasternum produced into a blunt process anteriorly, finely and very sparsely punctured in the middle, coarsely shallowly at the sides. Both sexes are alike except for the difference in the clypeus and the teeth of the front tibia.

Measurement: TL = 4.5 - 5.6 mm, BW = 2.8 - 3.1 mm, PL = 1.8 - 2 mm, PW = 2.6 - 2.8 mm, EL = 2.1 - 2.3 mm, HL = 1.2 - 1.3 mm, HW = 1.6 - 1.7 mm.

Material examined: 2 exs.(1♂, AIM-B_Co/Sc1000134 &1♀, AIM-B_ Co/Sc1000135), "India, Kerala, Kollam, Njarakkal, 9.X.2012, Coll. Priyadarsanan Dharma Rajan (PDR)".

Distribution: India: Uttarakhand, Bihar, Karnataka, Kerala.

Type: Natural History Museum, London (BMNH).

Remarks: This species was collected from the millipede *Trigoniulus corallinus* (Spirobolida: Trigoniulidae).

***Onthophagus coeruleicollis* Arrow**
(Plate 1, Image c)

Arrow, 1907: 430 (original description);
Arrow, 1931:184,185 (key & description);
Balthasar, 1963: 314 (monograph).

Diagnosis: Body broadly oval, highly convex; opaque above, shining beneath, head and pronotum deep blue or bluish- green, antennae and mouth organs bright yellow, elytra yellow with black transverse bands and spots; upper side covered with minute but numerous yellow hairs; head long and flat, clypeus produced into a blunt reflexed lobe, ocular lobes gently rounded, posterior part of the head semicircular; pronotum very convex, densely covered with fine oval granules, a slight smooth oblique impression at the base on each side; front angles of pronotum bluntly produced; elytra finely striate, intervals flat and finely granulate; pygidium strongly, fairly closely punctured; metasternal shield rather strongly, fairly closely punctured with its anterior edge vertical in the middle, sides of the metasternum moderately finely punctured.

Male: Clypeus densely punctured, vertex closely granulate; front tibia slightly elongate, teeth short, terminal spur very short and blunt. Female: Head closely granular; front tibia broad, terminal spur moderately long and pointed.

Measurement: TL = 6 - 8 mm, BW = 3.2 - 3.8 mm, PL = 2.4 - 2.6 mm, PW = 2.9 - 3.4 mm, EL = 2.3 - 2.7 mm, HL = 1.4 - 1.5 mm, HW = 1.6 - 1.9 mm.

Material examined: 4 exs. (1♂&1♀, AIM-B_ Co/Sc1000136- 137 from "India, Karnataka, Bangalore, Bannerghatta: Forest trail, 3.VI. 2010, Coll. SNK& PDR", from dead millipede;1♂, AIM-B_ Co/Sc1000138 from live millipede baited pitfall trap, "India, Karnataka, Bangalore, Srirampura, 26.IX.2011, Coll. PDR and SNK"and 1♂, AIM-B_ Co/Sc1000139, from millipede carcass "India, Andhra Pradesh, Maredumilli, 21. VII. 2015, Coll. Rajkamal Goswami (RG)".

Distribution: India: Karnataka,Maharashtra, Madhya Pradesh Odisha, Andhra Pradesh.

Type Depository: BMNH.

Remarks: This species was collected feeding on millipede carcass and from pitfalls baited with live millipede which released their defensive secretion.

***Onthophagus furcicollis* Arrow**
(Plate 1, Image d)

Arrow, 1931: 270, 276 (key & description);
Balthasar, 1963: 359 (monograph);
Scheuern, 1988 (description of female).

Diagnosis: Broadly oval, compact, convex; black, moderately shining, head slightly metallic; antennae, mouth parts and tarsi reddish, elytra with a red spot on shoulder and four others on posterior margin; upper surface with short setae; head smooth with

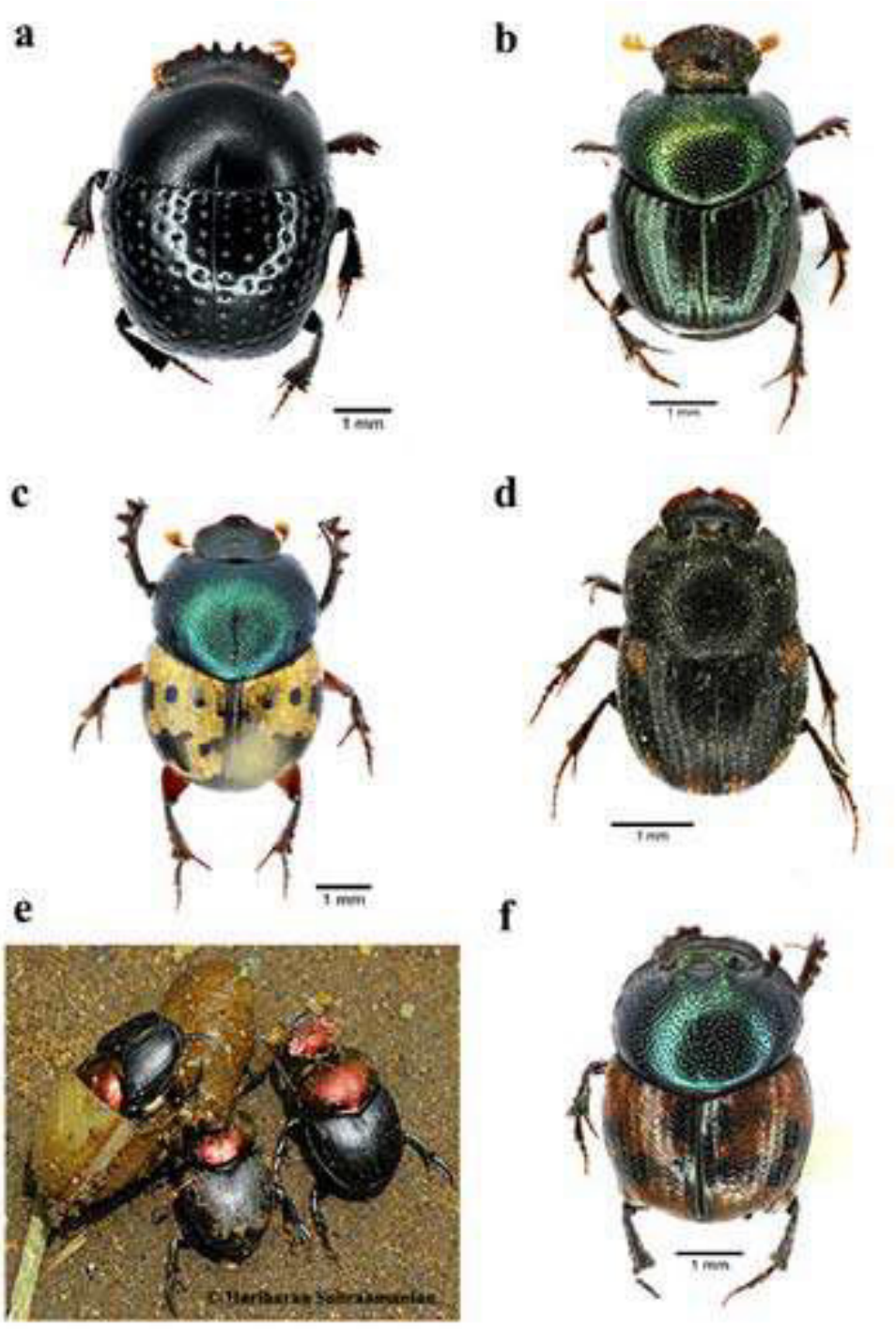

Plate 1. Image (a) ***Delopleurus parvus*** **(b)** ***Onthophagus arboreus*** **(c)** ***O. coeruleicollis*** **(d)** ***O. furcicollis*** **(e)** ***O. igneus*** **(f)** ***O. malabarensis***

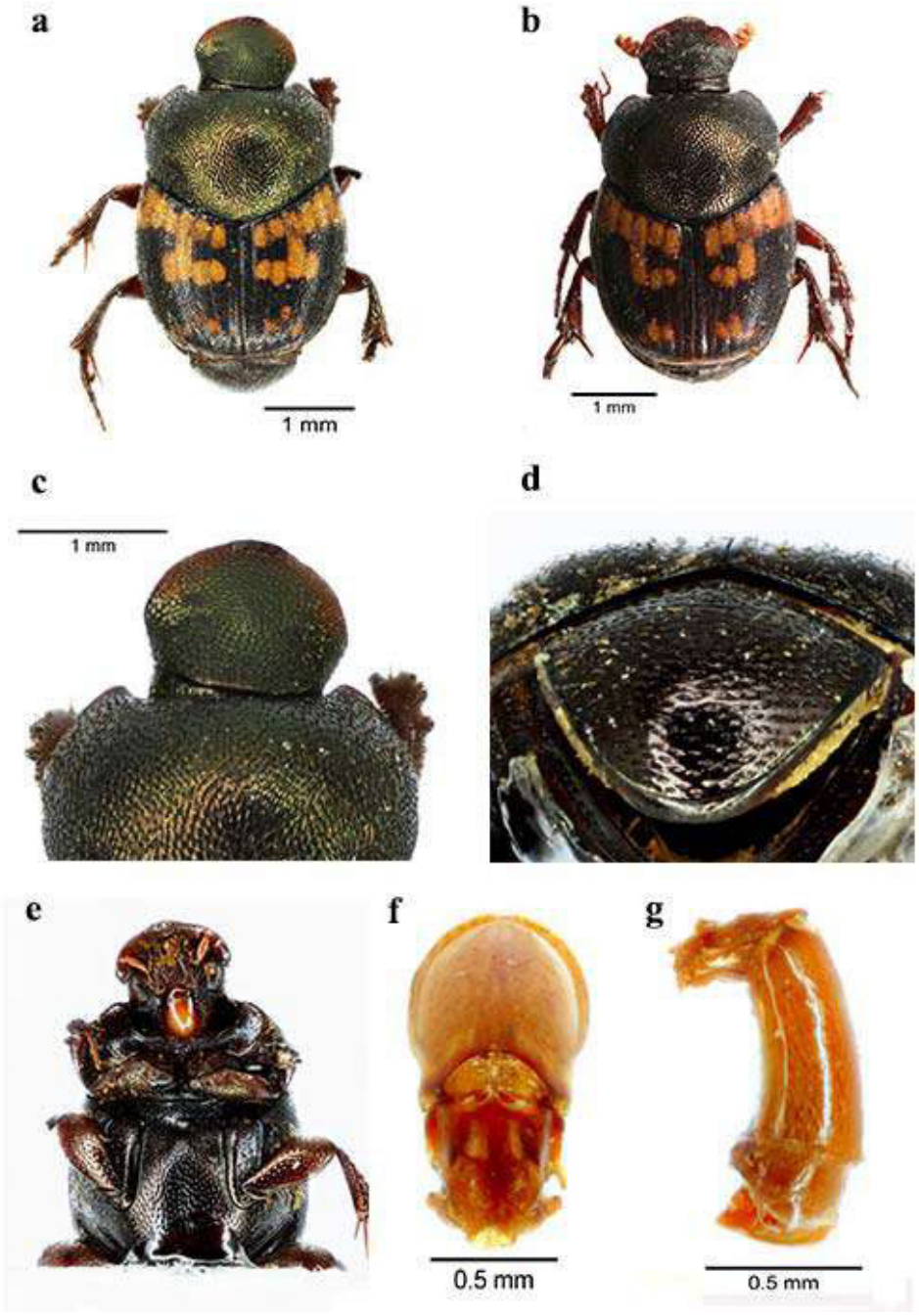

Plate 2. Image Holotype *Onthophagus jwalae* sp. nov.
(a) Dorsal habitus, male (b) Dorsal habitus, female; Male- (c) Head (d) Pygidium
(e) Ventral habitus; Genitalia- (f) apical view (g) lateral view

a few scattered puncture; clypeus bilobed; pronotum with close, large umbilicate punctures; elytral striae with chains of large annular punctures which are not contiguous, intervals asperately punctured; large annular punctures on pygidium; few scattered punctures on metasternal shield, sides of metasternum with fairly close annular punctures.

Male: Clypeal margin slightly bilobed in front with the lobes bluntly rounded; frontal carina absent, head with short straight horn between the eyes; front margin of pronotum with broad horizontal bifurcate process projecting over the head. Female: Clypeus sharply notched in front with lobes sharp, angulate, separated from forehead by a curved carina, there is a slightly elevated carina between the eyes; pronotum with a pair of tubercles behind the front margin.

Measurement: TL = 3.44 – 4.5 mm, BW = 2.06 - 2.5 mm, PL = 1.20 - 1.42 mm, PW = 1.9 - 2.15 mm, EL = 1.5 - 1.63 mm, HL = 0.82 - 0.9 mm, HW = 1.1 - 1.2 mm.

Materials examined: 3 exs. (1♂, AIM-B_ Co/Sc1000140 &1♀, AIM-B_ Co/Sc1000141), "India, Assam, Kohora, Kaziranga, N 26°34'46.47", E 93°24'27.73", Elev. 324ft., 27.X.2014" Coll: SNK"; 1♂ (Lectotype, BMNH(E) 1236994).

Distribution: India: Sikkim, Uttarakhand, Assam.

Type Depository: BMNH.

Remarks: This species was collected from a dead giant African snail, *Achatina fulica* Bowdich at a picnic spot near a stream in Kohora, Kaziranga, Assam.The specimens collected are smaller than the type (4 mm), the male specimen does not have horn and the process on the pronotum does not project over the head like in the type.

***Onthophagus igneus* Vigors**
(Plate 1, Image e)

Vigors, 1825 Zoological Journal 1: 409-418; 526-542 (description)

Diagnosis: Body broadly oval, deeply waisted, very convex; head flat, coarsely rugose, strongly angulate at the sides; black, with head (except anterior part of clypeus) and pronotum fiery crimson, pygidium deep blue or green, antennae bright orange-yellow; body thinly clothed with yellowish hair beneath; pronotum very convex, closely and evenly covered with not very minute oval granules, front angles blunt, lateral margins feebly sinuate in front, strongly behind, base obtusely angular in middle; elytra very finely striate, intervals flat, very minutely granular; pygidium very strongly and closely punctured; metasternum produced into a bluntly prominent process in front; almost smooth in the middle, fairly strongly punctured at sides.

Male: Clypeus little produced in front, narrowed, gently reflexed in middle, the posterior margin of the head is produced to a point in middle and curved gently upward; front margin of the pronotum with a small triangular excavation at the middle; club of antenna very large and broad.

Measurement: TL = 12.10 mm, BW = 6.67 mm, PL = 4.10 mm, PW = 6.35 mm, EL = 4.48 mm, HL = 3.28 mm, HW = 3.92 mm.

Material examined:1 exs. (1♂, AIM-B_ Co/Sc1000142), "India, Andhra Pradesh, Maredumilli, N 17°36'00.53", E 81°42'45.95" Elev. 1375 ft., July 2015, Coll. Ovee Thorat". 3 exs. Photographic evidence of the species feeding on snail was provided by Mr. Hariharan Subrahmanian from "India, Palakkad, Walayar, 09. VIII. 2012".

Distribution: India: Kerala, Karnataka, Tamil Nadu, Chhattisgarh.

Type Depository: BMNH

Remarks: This species was also collected from open cattle dung baits from BRT, Karnataka. In two other locations they were found feeding on unidentified dead snails.

***Onthophagus jwalae* Karimbumkara & Priyadarsanan sp. nov.**
urn:lsid:zoobank.org:act:54217E74-C226-42F3-AFC8-64A457948A5C
(Plate 2, Images a- g)

Description: Holotype, Male (Plate 2, Image a): Body oval, moderately convex, not very shining except for the pronotum and head which are slightly shining. Pronotum and head (Plate 2, Image c) bronzy- black, clypeus reddish; antennae and mouth organs reddish, antennal club yellow. Elytra black with yellow patches which extends from the 2nd interval to the 6th interval at the base and then curves upwards forming a hook which ends on the 7th interval thus leaving a black area encircled by yellow patch near the shoulder; yellow patch on the 4th interval extends almost half the length of elytra and bends towards the suture reaching upto 2nd interval; slight yellow streaks on 3rd to 6th intervals a little above the apical margin of the elytra. Head flat without any carina or horn; strong, large, close punctures on the vertex and ocular lobes, small, moderately close punctures on the clypeus. Clypeal margin parabolic, reflexed and slightly lobed in front. Pronotum closely granular with a smooth oblique area on both sides near the base. Pygidium (Plate 2, Image d) strongly convex, with rows of moderately close horizontally oval punctures. Metasternum (Plate 2, Image e) strongly and closely punctured, bluntly produced in front, small shallow punctures or rugosity in front angles. Sides of metasternum with scattered fine punctures with yellow setae. Both sexes look alike, except that the clypeus is transversely rugose in female (Plate 2, Image b), while it is moderately closely punctured in male.

Measurement: TL = 3.68 mm, BW = 2.12 mm, PL = 1.4 mm, PW = 1.84 - 1.88 mm, EL = 1.44 - 1.56 mm, HL = 0.8 mm, HW = 1 - 1.04 mm.

Genitalia (Plate 2, Images f, g): LP = 1.087 mm, Lp = 0.45 mm, BP = 0.434 mm, BpB = 0.37 mm, BpT = 0.33 mm.

Parameres 1/3 length of phallobase which is slightly curved, parameres almost straight above, joined from base to 3/4th its length, open in front with a thin rounded flap above, tip almost straight, broad, again joined in front as two rectangular lobes at the sides, with a sharp hook directed forward placed halfway from the base.

Type material: Holotype, male, "INDIA: Kerala, Njarackal, Kollam, N 08°56'29.6" E 076°36'20.5", Elev. 197 ft., 28. V. 2013, Coll. PDR" from millipede carcass of *Trigoniulus corallinus* (Spirobolida: Trigoniulidae), Reg. No. ZSI/ WGRS/ IR/ INV/ 7792a; Paratype, 1 female, same collection details as holotype, Reg. No. ZSI/ WGRS/ IR/ INV/ 7792b; deposited at ZSI-Calicut, Kerala, India.

Habitat: Collected on dead millipede from home garden.

Etymology:This species name '*jwalae*' comes from Sanskrit which means 'flame', and it is named so as there is a 'J or I'- shaped vertical marking on the elytra which is orangish- yellow or flame coloured.

Remarks: This species has been keyed out (Arrow, 1931: 184) to *Onthophagus coeruleicollis* Arrow, but *O. jwalae* is very different from the former and varies in the nature of pronotal granules, shape of the clypeus, size and colour; the fore tibia being longer in the former than the latter.

***Onthophagus malabarensis* Boucomont**
(Plate 1, Image f)

Boucomont, 1919: 314 (original description);
Arrow, 1931: 345 (key & description);
Balthasar, 1963: 429 (monograph)

Diagnosis: Female: Deep green or coppery, head and pronotum brighter green or blue, elytra bright orange, sutural line black, irregular post-median bar extending obliquely from side to side; abdomen and pygidium black, tarsi, antennae and mouth-organs reddish, body broadly oval, compact and convex, with a thin clothing of short erect yellowish setae; head not wide, clypeus slightly bilobed; transversely rugose, separated by gently curved carina from the well- punctured forehead, there is a straight carina behind the eyes; pronotum moderately strongly, evenly and closely punctured; front angles not very sharp; has a blunt tubercle in front on each side in the middle; elytra finely striate, intervals flat and finely but distinctly punctured in double series; pygidium shining and fairly strongly punctured; metasternum sparingly, unevenly and fairly strongly punctured.

Measurement: TL = 4 - 5 mm, BW = 2.5 - 3.08 mm, PL = 2.13 mm, PW = 2.74 mm, EL = 2.07 mm, HL = 1.12 mm, HW = 1.51 mm.

Material examined: 1 ex. (♀,AIM-B_ Co/ Sc1000143), "India, Kerala, Eranakulam, Bhoothathankettu, 22.X. 2010, Coll. SNK".

Distribution: India: Uttar Pradesh, Maharashtra, Kerala.

Type: MNHN.

Remarks: This species is a carrion feeder and was found feeding on dead millipede.

***Onthophagus pithankithae* Karimbumkara & Priyadarsanan sp. nov.**

urn:lsid:zoobank.org:act:6A4D8BEA-402A-4C97-A3C1-AF72369BA1B6

(Plate 3, Images a- g)

Description: Holotype, Male (Plate 3, Image a): Oval, deeply waisted, moderately convex. Body black, legs reddish black, mouthparts, antennae and tarsi reddish; head (Plate 3, Image c) and pronotum metallic green and elytra black with yellow patches - one on the sixth and seventh striae towards the angles of shoulder; an angulate band that extends from half of the outer margin to the inner margin, but does not touch the suture and continues to the elytral base between 3rd and 4th striae; a yellow patch near the inner margin at the tip of the elytra between first and fourth striae; and another one at the tip of the elytra starting after the fifth striae and extending to the outer margin in continuity with the middle band. Body with scattered pale setae; head shining with scattered strong punctures separated by a curved carina from the clypeus and there is a straight carina between the eyes. Clypeus bidentate, excised in front and the sides rounded, smooth in the middle, margin reflexed. Ocular lobes gently rounded with scattered punctures. Pronotum moderately closely and strongly punctured with scattered inconspicuous punctures in between the large punctures; front angles produced, rather blunt, lateral margins straight in front, strongly rounded in the middle, sinuate behind and gently rounded at the base. Elytra moderately strongly striate, punctures on striae not close to each other; intervals shining with punctures arranged in two rows closer to the striae. Pygidium (Plate 3, Image d) shining, deeply, uniformly and not very closely punctured. Metasternal shield (Plate 3, Image e) smooth in the middle with strong scattered punctures at the sides which are closer towards the front. Sides of the metasternum strongly but not very closely punctured. Fore-legs slender with four teeth, two in front very large and the fourth very small compared to the third tooth. Spur sharp, slender and slightly curved towards the tip.

Male: Horns absent. Pronotum without tubercle, clypeus smooth and shining with scattered punctures. Carina between the eyes and that which separates clypeus and the vertex are not very prominent. Clypeus strongly punctured at the sides, punctures finer towards the margin.

Measurement: TL = 2.8 - 3.28 mm, BW = 1.6 - 2.08 mm, PL = 0.92 - 1.32 mm, PW = 1.32 - 1.72 mm, EL = 1.04 - 1.28 mm, HL = 0.64 - 0.8 mm, HW = 0.72 - 0.92 mm.

Genitalia: (Plate 3, Images f, g) LP = 0.76 mm, Lp = 0.46 mm, BP = 0.304 mm, BpB = 0.304 mm, BpT = 0.065 mm.

Phallobase longer than parameres, almost double its length, slightly curved, parameres short, sharp and hooked at the tip, joined at the base, open in the middle, again meets in front but the tips apart giving it a bidentate appearance.

Female (Plate 3, Image b): Clypeus more strongly and rugosely punctured with smooth area in the middle. Frontal carina strongly curved, carina between the eyes straight and elevated; two slightly pointed tubercles present on pronotum which are not connected.

Type material: Holotype, male, "INDIA: Karnataka, Bannerghatta, Forest trail, Guddayyanadoddi, N 12°43.233', E 077°33.576', Elev. 905 m, 3. VI. 2010. Coll: SNK & PDR" from carcass of millipede *Phyllogonostreptus nigrolabiatus* (Spirostreptida: Harpagophoridae), Reg. No. ZSI/ WGRS/ IR/ INV/ 7793a, Paratype, 1 female, same collection

details as holotype, Reg. No. ZSI/ WGRS/ IR/ INV/ 7793b; deposited at ZSI-Calicut, Kerala, India.

Habitat: Found feeding on dead millipedes near the Forest trail camping ground.Vegetation type is tropical moist mixed forest.

Etymology: The species name '*pithankithae*' means 'yellow marked' in Sanskrit and this species is named so as their elytra has yellow patterns on it.

Remarks: *Onthophagus pithankithae* is closer to *O. ludio* Boucomont. The major differences between these two species are the former is smaller in size, the clypeus is smooth with few punctures. The male without horn (can be a minor male), elytra simply punctured and metasternum smooth in the middle; while *O. ludio* is larger, clypeus rugose, head is produced into a triangular lamina behind, the apex of which extends to a short pointed horn curving upwards; elytra is with aciculate punctures, and metasternum strongly punctured in the middle.

***Onthophagus pygmaeus* (Schaller)**
(Plate 4, Image a)

Scarabaeus pygmaeus (Schaller), 1783: 239 (original description);
Onthophagus pygmaeus Fabricius, 1792: 44 (description);
Arrow, 1931: 209 (key & description);
O. tigrinus Castelnau, 1840: 87 (synonym);
O. lucens Walker, 1858 (synonym);

Description: Shining blue, green, coppery or golden above, lower surface nearly black, elytra bright yellow, with black transverse bars and spots; body oval and convex, clothed above and beneath with pale setae; head not broad, sides rounded before the eyes, clypeus bilobed in front separated from forehead by a curved carina and a similar straight carina behind the eyes; pronotum rather strongly and closely punctured in its basal part, puncture changing to granules anteriorly; front angles rather sharp; elytra finely striate, intervals flat and fairly strongly punctured; pygidium very strongly and closely punctured with a clothing of long, close pale hairs; metasternal shield rather strongly sparingly punctured and sides of metasternum more closely.

Male: Head very smooth, shining, bears only a few scattered punctures; clypeus little produced, narrowed, strongly reflexed in front. Anterior margin of pronotum very smooth, a little hollowed on each side with two blunt lobes in front; front leg very long, tibia slender, feebly curved with very short and distant tooth, terminal spur long and curved. Female: Clypeus short and coarsely rugose; pronotal front margin with a broad, bituberculate prominence behind. Front tibia broad with rather strong external teeth.

Measurement: TL = 3.92 - 5.4 mm, BW = 2.5 - 3.36 mm, PL = 1.73 - 2.41 mm, PW = 2.24 - 3.08 mm, EL = 1.62 - 2.24 mm, HL = 0.95 - 1.23 mm, HW = 1.18 - 1.73 mm.

Material examined: 8exs. (1♂, AIM-B_Co/ Sc1000144), "India, Karnataka, Bannerghatta, Forest trail, Guddayyanadoddi, N 12°43.233', E 077°33.576', Elev. 905m, 3. VI. 2010. Coll: SNK & PDR", (1♂ & 1♀, AIM-B_ Co/Sc1000145- 146), "India, Kerala, Boothathankettu, 20.VII. 2011, Coll. SNK", (2♂&2♀, AIM-B_Co/Sc1000147- 150), "India, Kerala, Ernakulam, Valanthakkad island, 28.X. 2012, Coll. PDR"; 1♂, BMNH(E) 1237082).

Distribution: India: Kerala, Karnataka, Srilanka.

Type: In the Halle Museum?.

Remarks: Out of the seven specimens collected three were from millipede carcass while four were from goat droppings. This species was also collected from a dead lizard (Arrow, 1931).

***Onthophagus* (*Parascatonomus*) *rudis* Sharp**
(Plate 4, Image b)

Sharp, 1875: 58 (original description);
Boucomont, 1914: 271 (list);
Boucomont et Gillet, 1921: 41 (list);
Boucomont, 1924: 669 (list);
Boucomont, 1925: 153 (list);
Arrow, 1931: 184, 185 (keys & description);
Balthasar, 1935: 329 (monograph);
Paulian, 1945; 88, 102 (list);

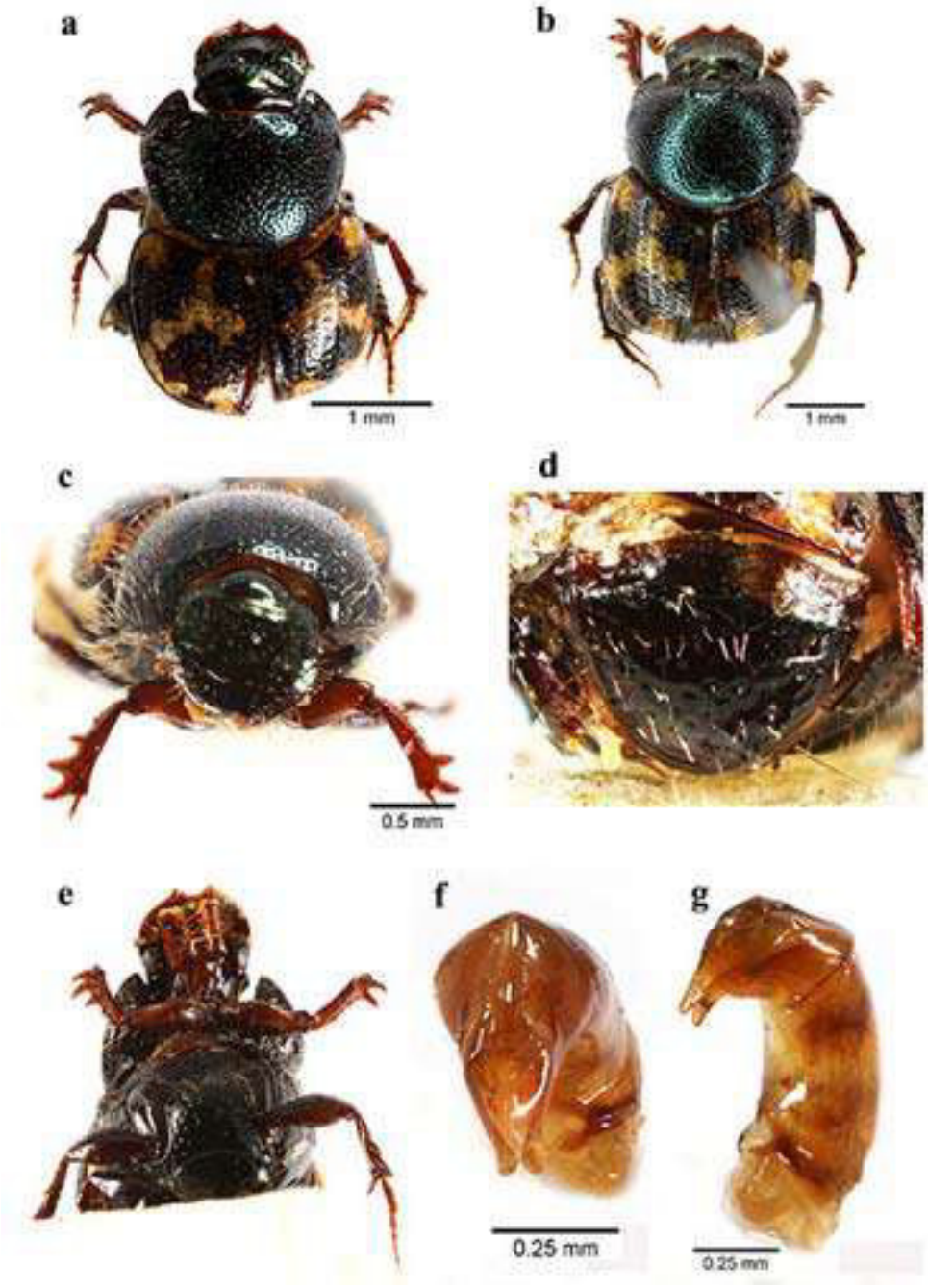

Plate 3. Image Holotype *Onthophagus pithankithae* sp. nov.
(a) Dorsal habitus, male (b) Dorsal habitus, female; Male (c) Head (d) Pygidium
(e) Ventral habitus; Genitalia- (f) apical view (g) lateral view

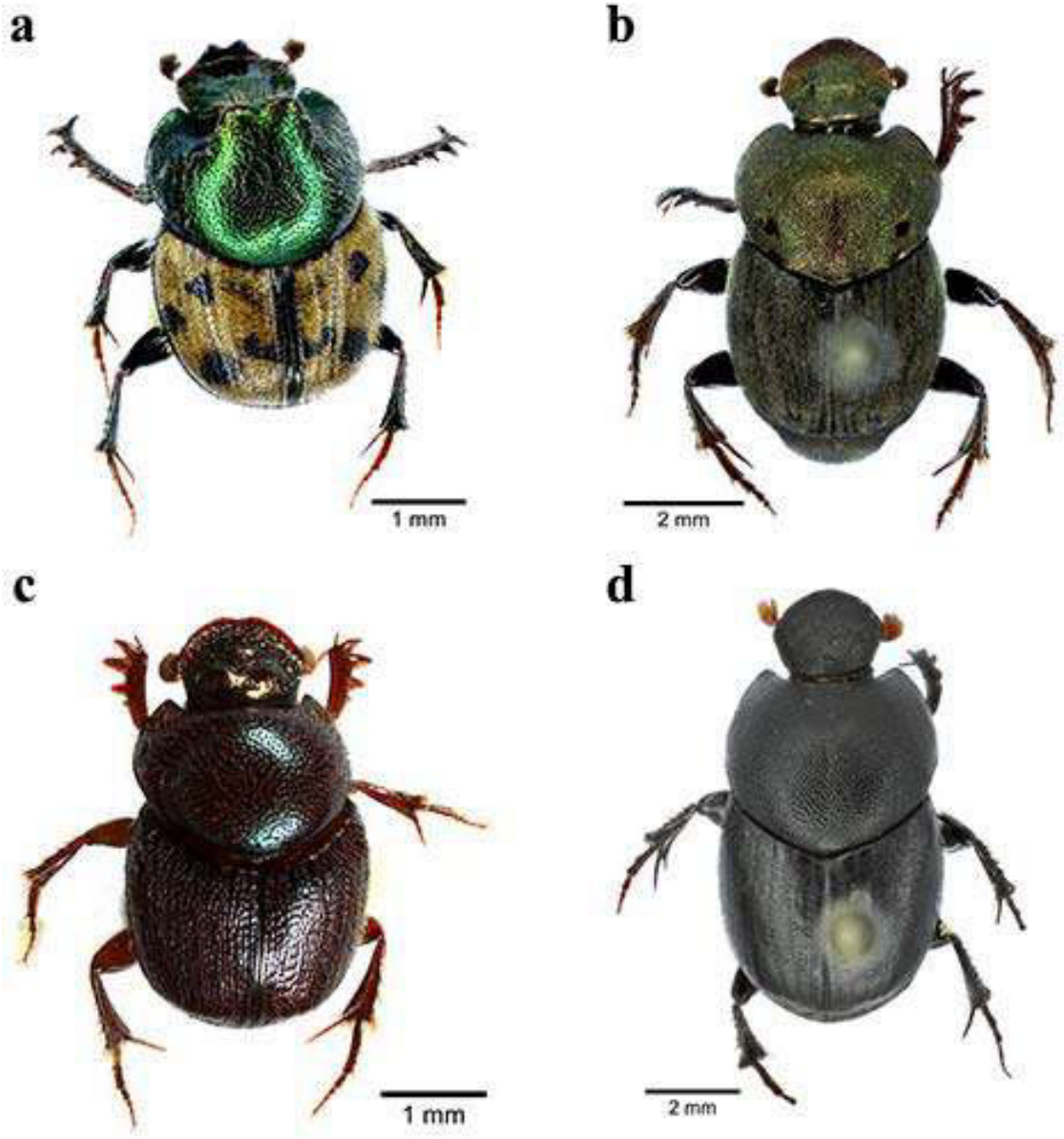

Plate 4. Image (a) ***Onthophagus pygmaeus*** **(b)** ***O. rudis*** **(c)** ***O. tritinctus*** **(d)** ***O. vultur***

Balthasar, 1963: 505 (monograph).
- *aper* Sharp, 1875: 59 (synonym);
- *foveolatus* Harold, 1877: 68 (synonym);

Diagnosis: Body slightly metallic, dark greenish brown, shining above, opaque below, covered with greyish setae; oval, not broad, very convex, strongly constricted at the waist. Head flat, closely and evenly rugulose, frontal carinae absent, clypeus little produced, blunt, reflexed in the middle; pronotum densely covered with fine oval granules, with a small smooth pit on each side near the hind margin and a slight depression in the middle of the base; front angles blunt, base produced backward and obtusely angular in the middle; elytra finely striate, intervals flat, closely covered with minute elongate granules; pygidium fairly closely covered with fine granules; metasternal shield evenly, fairly strongly punctured, narrowed and almost vertical in front; sides of metasternum more strongly and closely punctured.Male and female are alike except that the females are larger, have a broader head, front tibia has blunt teeth.

Type: MNHN.

Measurement: TL = 5 - 8 mm, BW = 3 - 4.5 mm, PL = 2.6 - 3.12 mm, PW = 3.3 - 3.9 mm, EL = 2.5 - 3.12 mm, HL = 1.5 - 2 mm, HW = 1.88 - 1.12 mm.

Material examined: 3 exs. (1♂&1♀, AIM-B_ Co/ Sc1000151- 152), "India, Kerala, Boothathankettu, 22.X. 2010, Coll. SNK" and (1♀, AIM-B_ Co/ Sc1000153), "India, Kerala, Ernakulam, Kunnathunad Taluk, Iringole kaavu, 9.VII.2011, Coll. SNK & PDR".

Distribution: India: Karnataka, Assam, Madhya Pradesh, Kerala; Mayanmar; Philippines; Indonesia; Sunda Islands; Java; Sumatra; Borneo; Nias; Thailand; North Vietnam; China.

Remarks: Out of the 3 specimens of *Onthophagus rudis* , those collected from Boothathankettu were found feeding on a live millipede and the other from a millipede carcass. Both the millipedes belongs to genus *Phyllogonostreptus.*

***Onthophagus tritinctus* Boucomont**
(Plate 4, Image c)

Boucomont, 1914: 217 (original description);
Arrow, 1931:263,266 (keys & description);
Balthasar, 1935: 338 (monograph);
Balthasar, 1963: 564 (monograph);
Paulian, 1945:89, 127 (description).

Diagnosis: Body black shining; head fiery- red, pronotum blue or green, antennae and mouth organs yellow, tarsi red, body broadly oval, compact, convex, clothed with yellow setae; head fairly strongly dilated at the sides, clypeal margin rounded, very feebly excised in the middle; clypeus separated by short transverse carina from the strongly but not closely punctured forehead which bears between the eyes a pair of blunt tubercles; pronotum evenly moderately strongly and closely punctured, front angles sharp; elytra finely striate, 7th stria straight, parallel with the 6th intervals fairly closely, not very finely punctured; pygidium fairly strongly and closely punctured; metasternal shield bears scattered, not very fine punctures, sides of the metasternum rather finer and more numerously punctured. Male: Clypeus shining, not closely rugose. Female: Clypeus closely rugose and not shining.

Type: MNHN.

Measurement: TL = 3.5 - 4.5 mm, BW = 1.98 - 2.5 mm, PL = 1.12 - 1.41 mm, PW = 1.72 - 2.02 mm, EL = 1.46 - 1.6 mm, HL = 0.77 - 0.86 mm, HW = 1.07 - 1.16 mm.

Material examined: 2 exs., (1♂, AIM-B_ Co/ Sc1000154), "India, Karnataka, Bangalore, Bannerghatta: Forest trail , 3.VI. 2010, Coll. SNK & PDR"; (1♂, AIM-B_ Co/Sc1000155), "India, Kerala, Njarackal, Kollam, 28. V. 2013, Coll. PDR".

Distribution: India: Maharashtra, Tamil Nadu, Karnataka; Srilanka, China.

Remarks: Both specimens were collected from millipede carcass.

***Onthophagus tharalithae* Karimbumkara & Priyadarsanan sp. nov.**

urn:lsid:zoobank.org:act:1D8A8886-7CA6-4724-8B1A-37C7DE766D1C

(Plate 5, Images a - g)

Description: Holotype, Male (Plate 5, Image a): Oval, moderately convex, slightly shining, blackish-brown; legs, antennal stalk and clypeus reddish, mouthparts and antennal club yellow; clypeal margin almost straight in front, lateral margins wavy; head (Plate 5, Image c) densely, unevenly punctate with a smooth, frontal carina represented by afeeble line and another straight, slightly elevated carina between the eyes. Pronotum densely, moderately strongly punctate, sides rounded in front, distinctly sinuate posteriorly, base rounded, front angles not very sharp; elytra with a single reddish spot on shoulder upon 6th and 7th intervals and similar spots spread on 4th to 6th in the apex. Elytral striae not very deep, striae moderately closely punctured, elytral intervals minutely, unevenly asperately punctured; Pygidium (Plate 5, Image d) strongly, moderately closely punctured; metasternal shield (Plate 5, Image b) smooth in the middle with uneven punctures at the sides, sides of metasternum with stronger punctures.

Measurement: TL = 3.5 - 4.54 mm, BW = 2 - 2.63 mm, PL = 1.68 mm, PW = 2.41 mm, EL = 1.96 mm, HL = 0.95 mm, HW = 1.46 mm.

Genitalia (Plate 5, Images e- g): LP = 1.05 mm, Lp = 0.65 mm, BP = 0.46 mm, BpB = 0.49 mm, BpT = 0.162 mm.

Phallobase longer than parameres, slightly curved; parameres triangular in appearance from above, joined at the base till the front end where they slightly superpose and elevates, then it curves forward and down, bifurcates and diverges towards the tip.

Female: Unknown

Type Material: Holotype, male, "INDIA: Assam, Golaghat, Kohora, N 26°34'46.47", E 93°24'27.73", Elev. 324 ft., 27.X.2014. Coll: SNK from a dead giant African snail (*Achatina fulica* Bowdich). Reg. No. ZSI/ WGRS/ IR/ INV/ 7794; deposited at ZSI-Calicut, Kerala, India.

Habitat: Collected on a dead snail which was found near a stream feeding along with a few *O. furcicollis*.

Etymology: This species gets the name *tharalithae* from Sanskrit which means undulating or wavy. It is named so, as the clypeus margin is undulating.

Remarks: *Onthophagus tharalithae* is similar to *O. pauliani* Frey in its size and the elytra having spots but differs in the clypeal margin being truncate and undulate, the antennal club being yellow; the front angles of pronotum being sharper and the red spots present only near the shoulder, while in *O. pauliani* clypeal margin is slightly emarginate, antennal club is dark and red spots are present at the base of 2nd and 4th striae in addition to the shoulder.

***Onthophagus vultur* Arrow**

(Plate 4, Image d)

Arrow, 1931: 197 (original description);
Balthasar, 1963: 588 (monograph)

Diagnosis: Black, opaque above, antennae and mouth- organs red, clothed with extremely minute inconspicuous setae above and fairly thick hairs at sides below; oval, very convex; head flat, closely punctate- rugose, sides bluntly angulate; clypeus produced to an obtuse distinct angle in front; fronto - clypeal carina absent, forehead with a slight median depression and a slight transverse elevation behind; pronotum closely and evenly covered with granules, front angles not very blunt; elytra lightly striate, intervals flat, bearing numerous minute granules; pygidium strongly, closely, partly confluently punctured; metasternal shield rather strongly punctured except in the middle where punctures are fine; sides of the metasternum strongly, closely punctured. Both sexes look alike.

Measurement: TL = 8 - 8.7 mm, BW = 4 - 4.7 mm, PL = 3.2 mm, PW = 4.5 mm, EL = 3.4 mm, HL = 1.6 - 1.7 mm, HW = 2.3 mm.

Plate 5. Image Holotype, Male- *Onthophagus tharalithae* sp. nov.
(a) Dorsal habitus (b) Ventral habitus (c) Head (d) Pygidium; Genitalia (e) lateral view (f) apical view (g) ventral view

Materials examined: 3 exs. (1♂, AIM-B_ Co/Sc1000156, 2♀, AIM-B_ Co/Sc1000157- 158), "India, Andhra Pradesh, Maredumilli, N 17°36'00.53", E 81°42'45.95" Elev. 1375 ft., Coll. RG".

Distribution: India: Maharashtra, Karnataka, Andhra Pradesh.

Type Depository: BMNH.

Remarks: *Onthophagus vultur* was originally described by Arrow (1931) based on a specimen collected by H.M. Lefroy found feeding on a dead locust from Igatpuri (now in Maharashtra state) and H.E. Andrews from Belgaum (now in Karnataka). RG collected 3 individuals of this species while feeding on a dead millipede. This is the rediscovery of the species after 85 years of its original description.

DISCUSSION

While most Scarabaeinae depend on mammalian dung or carcasses for feeding and breeding, many of them also take to unconventional resources like carcasses of invertebrates, decaying fruits and fungus. Hitherto absence of *O.vultur* from any later collections after its original description, points to specialisation of atleast some species to invertebrate carcasses. The reason why many of these species were rarely collected from dung bait traps can be attributed to their necrophagous or saprophagous behaviour or their affinity to specific cues, like the defensive secretion of the millipede. A single specimen of *O. coeruleicollis* was retrieved from a pitfall trap that was baited with live millipede. Even though they got easily trapped in baits with millipedes they were never found attracted to dung baited pitfall traps. More observations and studies needto be conducted to check whether the carrion specialist *O. rudis* (Hanski, 1983; Kikuta *et al.,* 1997, Brühl and Krell, 2003) predates and kills the millipede or were they just attracted to its defensive secretion (Kon *et al.,* 1998).

Most of dung beetles those feed on millipede carcasses were found to have similar morphological characteristics like absence of horns, small size, granular pronotum and in some case a lobed clypeus, which can be used in cutting open or prying through the millipede body. Their adaptation to sense the defensive secretion of millipedes are advantageous to these beetles as the quinonous secretions helps them in avoiding other necrophagous competitors and access to the fresh kill before it starts decomposing.

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Suppression of growth and endopeptidases of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) infesting coconut using proteinase inhibitors

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ABSTRACT: Investigations on luminal proteinases of grubs of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) infesting coconut revealed presence of two endodpeptidases *viz*., trypsin (BA*p*NA-ase activity) and elastase-like chymotrypsin (SAAPL*p*NA-ase activity) in all stages of larval development. Highest activity of these proteinases coincided with the active feeding stage (mid-larval stage) of the insect. Aprotinin 50 µg, Soybean Trypsin Inhibitor (SBTI) 50 µg and Phenyl Methyl Sulphonyl Fluoride (PMSF) 1700 µg inhibited trypsin activity of *R. ferrugineus* by 77.4%, 63.1% and 55.9%, respectively. Serine proteinase inhibitors *viz*., aprotinin (50 µg), SBTI (50 µg) and PMSF (1700 µg) had a marginal reduction of elastase - like chymotrypsin activity of *R. ferrugineus* by 32%, 14% and 11%, respectively suggesting the serine nature of the proteinase. *In vivo* bioassay of 250 µM aprotinin on coconut petiole method using early stage grubs of *R. ferrugineus* indicated a significant weight loss of 18.9% due to incorporation of serine proteinase inhibitor, aprotinin in a period of 120 h. Possibility of using serine proteinase inhibitor, aprotinin in the management of *R. ferrugineus* was suggested.

KEY WORDS: *Rhynchophorus ferrugineus,* gut proteinases, proteinase inhibitors, aprotinin, soybean trypsin inhibitor

INTRODUCTION

Among the various insects that affect the coconut production, red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) is the key pest of economic significance. *R. ferrugineus*, a concealed tissue borer, is a lethal pest of palms and is reported to attack 17 palm species world wide. Currently, the pest is reported in 15% of the coconut-growing countries and in nearly 50% of the date palm-growing countries (Faleiro *et al*., 2006). Infested palms, if not detected early and treated, often die. However, palms in the early stages of attack respond to chemical treatment with insecticide. The major components of the Integrated pest Management (IPM) programmed for RPW in coconut are surveillance, maintaining plant and field sanitation, preventive chemical treatment of wounds, filling the leaf axils of young palms with a mixture of insecticide and sand, curative chemical treatment of infested palm, cutting and burning of severely infested palms, trapped adults using food attractants (Rajan and Nair, 1997; Faleiro *et al*., 2006).

It is well established that proteolytic enzymes in insect gut are primarily responsible for the digestion of plants proteins. Since insects are unable to synthesize a number of amino acids, they depend on digestive proteinase and plant proteins to meet

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their nutritional requirements (Bernays and Woodhead, 1984). Proteins are digested in the insects gut by enzymes that are active in fairly alkaline *p*H (Lepidoptera) to slightly acidic *p*H (Coleoptera) and serine proteianses account for 95% digestive activity (Applebaum, 1985). Digestive enzymes such as serine proteases, cysteine proteases and other peptidases excreted into the lumen of the larval midgut are responsible to the food protein digestion (Gatehouse *et al*., 1997) and have been considered as to be potential targets for the insect pest management (Jongsma *et al*., 1995).

Plant-derived proteinase inhibitors (PI) are of a particular interest because they are part of the plant natural defense system against insect predation. Previous studies on the effect of dietary proteinase inhibitor either artificially introduced into defined diets or already present in plant tissues, have shown that these PI can be detrimental to growth and development of a wide range of insects (Ryan, 1990; Hilder *et al*., 1987). Proteinase inhibitors can bind with key digestive proteases of insects feeding on plants, disrupting their digestion and reducing growth and survival (Gatehouse *et al*., 2000). It might be possible to control larval stages of *R. ferrugineus* by identifying potential targets like proteinase inhibitors and also unravel the potential of proteinase-inhibitors from legumes for manipulation in management of RPW.

Disruption of protein digestion by proteinase inhibitors represents an alternative approach to pest management in a world dominated by chemical pesticides which besides increasing the production cost, cause environmental hazards. This approach requires a thorough understanding of the biochemical properties of the proteases from the gut homogenate, characterization of these endopeptidases particularly trypsin (EC 3.4.21.4) and elastase-like chymotrypsin (EC 3.4.21.1) in relation to developmental stages and understanding the way it reacts with classical proteianse inhibitors such as soybean trypsin inhibitor and aprotinin.

Keeping this in view, a study on the endopeptidase activities *viz*., trypsin and elastase-like chymotrypsin) of *R. ferrugineus* grubs and its interaction with proteianse inhibitors has been attempted. In the present study, assay conditions of both the endopeptidases were optimized and the effect of metal ions and inhibitors on trypsin and elastase-like chymotrypsin activity of the crude midgut homogenate from *R. ferrugineus* was determined. Characterization of both endopeptidases in relation to developmental stages was analyzed.

MATERIALS AND METHODS

Insect source: Grubs of *R. ferrugineus* used in this study were collected from infested coconut palms in the Research Farm of ICAR Central Plantation Crops Research Institute (CPCRI), Regional Station, Kayamkulam, Alappuzha district, Kerala located at 9°48' N latitude and 76°19'E longitude at an altitude of 3.05 m above Mean Sea Level. Field strains of *R. ferrugineus* were maintained on succulent coconut crown pieces (cabbage) placed in plastic container at 27 ± 2°C and 70 ± 10% relative humidity that was standardized as optimum rearing condition for the pest. Coconut cabbages were replaced on every alternate day to avoid microbial contamination of the fresh plant substrates used as feeding media. *R. ferrugineus* grubs of various stages *viz*., early-instar (<2 g), mid-instar (2-4 g) and late-instar (>4 g) coinciding the physiological stages of pest were used in the study. Two prominent endopetidases *viz*., trypsin (BA*p*NA-ase activity) and elastase-like chymotrypsin (SAAPL*p*NA-ase activity) were investigated.

Chemical source: Substrate for trypsin-like proteinase N-Benzoyl L-arginine *p*-nitroanilide (BA*p*NA) and elastase-like chymotrypsin Succinyl-ala-ala-pro-leu-*p*- nitroanilide (SAAPL*p*NA) and protease inhibitors such as aprotinin, soybean trypsin inhibitor and phenyl methane sulphonyl fluoride (PMSF) were purchased from Sigma-Aldrich Chemical Company (St. Louis, USA). All other chemicals / reagents obtained from Sisco Research Laboratories, Mumbai were of analytical grade of superior quality. Spectrophotometric measurements were recorded using Cary 50 UV-Visible single

beam spectrophotometer linked to desktop computer.

Preparation of gut extracts: *R. ferrugineus* larvae were sampled two days after head-capsule slippage when the active feeding behaviour of the insect pest was observed. Three different stages of the test insect (early, mid and late-instar) coinciding the physiological stages of development and appropriate age were selected for extraction of gut. Larvae were cold (-20°C) anesthetized for 10 minutes and individual gut was dissected out in insect saline. The dissected gut was isolated free of fat tissues, dehydrated using filter paper, weighed and taken out in Eppendorf tube with 20 mM Tris-HCl, *p*H 8.0. The guts were homogenized using a plastic homogenizer in 1000 µl of 20 mM Tris-HCl, *p*H 8.0. Buffer is added in order to maintain the desired *p*H and thereby maintenance of intact enzyme activity. Homogenates were clarified to remove particulate matter by centrifugation (Hereaus centrifuge) at 12000 rpm for 15 minutes at -4°C. Supernatants were transferred to clean tubes and stored at -20°C for use in peptidase enzyme assay.

Enzyme assay conditions: The trypsin assay condition for the crude gut extract were standardized using 50 mM Sodium citrate buffer *p*H 6.0, 5.0 mM Tris-HCl buffer *p*H 7.0, 8.0, 9.0 and 100 mM Sodium bicarbonate buffer *p*H 10.0, 11.0, temperature ranging from 37-50°C and incubation time ranging from 20-40 minutes using BA*p*NA (1 mM) as substrate. Similarly assay condition for elastase-like chymotrypsin was standardized using buffers ranging from *p*H 6-11, temperature range from 37-55°C and incubation time from 20-50 min using SAAPL*p*NA (1 mM) as substrate. Assays were performed according to Burgess *et al*. (2002) with slight modification in a reaction volume of 4.0 ml comprising of 25 µl of crude gut homogenate, 275 µl water, 100 µl of 1mM BA*p*NA /1mM SAAPL*p*NA, 3200 µl of buffer and 400 µl of stopping reagent. Reaction was started by the addition of 25 µl of crude gut homogenate to the buffered substrate solution and then incubated at relevant temperature. The enzymatic reaction was stopped by the addition 400 µl of 30% acetic acid (stopping reagent) after the required period of incubation as outlined by Josephrajkumar *et al*. (2005). All assays were carried out in duplicate and blanks were used to account for spontaneous breakdown of substrates. Controls were incubated similarly, but acetic acid was added at the beginning of each assay. The peptidase as well as elastase-like chymotrysin activities were determined by the amount of *p*-nitroaniline (*p*NA) released from the substrate and were measured at 405 nm (Thangam and Rajkumar, 2002). The activity was expressed as nanomoles of *p*NA released per minute per gram of the gut tissue (*Molar extinction coefficient of pNA is 9500 M^{-1} cm^{-1})*. Total protein in the crude gut extract was determined according to the method of Lowry *et al*. (1951) using bovine serum albumin as standard and expressed as mg g^{-1}. Specific activity was represented as activity per mg protein.

Effect of metal ions on peptidase / elastase-like chymotrypsin activities: In order to determine the optimum metal ion required in enzyme assay, 25 µl of insect gut extract was diluted to 400µl using distilled water and mixed with 3200?l of 50 mM Tris-HCl buffer (*p*H 9.0) in case of peptidase assay and 50 mM Tris-HCl buffer (*p*H 8.0) for elastase-like chymotrypsin consisting of different metal ions $CaCl_2$, $MgSO_4$, $ZnSO_4$, Na_2SO_4, $CuSO_4$ and $HgCl_2$ (20 mM) in separate tubes. After 10 minutes of incubation, 100µl of 1mM BA*p*NA / 1mM SAAPL*p*NA was added and mixed thoroughly. The mixture was incubated at 40°C for 25 minutes and the assays were performed in duplicate as indicated above.

Enzyme activities on different instars of *R. ferrugineus:* In order to determine the enzyme activities in different larval instars, 25 µl of gut extracts of different instars (early, mid and late instars) of *R. ferrugineus* larvae were taken in test tubes and the volume was made up to 400 µl using distilled water. To this 3200µl of 50 mM Tris-HCl buffer (*p*H 9.0) with 20mM Na_2SO_4 was added in case of peptidase assay and 50 mM Tris-HCl buffer (*p*H 8.0) with 20mM $CaCl_2$ for chymotrypsin

assay. The reaction mixture was incubated at 40°C for 25 minutes in water bath after addition of relevant substrate and proceeded as above.

Effect of inhibitors on enzyme activities: Inhibition assays were carried out using aprotinin (0-50 µg), and soybean trypsin inhibitors (0- 50µg) and phenyl methyl sulphonylfluoride (0-1700 µg), which are the classical inhibitors of serine protease. Different amounts of these inhibitors were added to the reaction mixture of 4.0 ml comprising of 25 µl of crude gut homogenate, 175-275 µl water, 100 µl of 1mM BA*p*NA, 3200 µl of 50 mM Tris-HCl buffer (*p*H 9.0) with 20mM Na_2SO_4 and 400 µl of stopping reagent. In case of chymotrypsin assay 50 mM Tris-HCl buffer (*p*H 8.0) with 20mM $CaCl_2$ was used. The reaction mixture was incubated at 40°C for 25 minutes as mentioned in earlier experiments.

Feeding bioassay: Laboratory experiment was conducted at room temperature (28-30°C) in 100 ml plastic cups filled with 40-50 g of fresh skin-pealed coconut petiole. 1 ml of 250 µM of aprotinin was painted on the coconut petiole and fed to seven early larvae of *R. ferrugineus* maintained in separate containers. Similarly control was maintained and larvae fed on coconut petiole devoid of aprotinin. Initial larval weight and weight gain after 120 h of each larvae was recorded. All data were compared with Student's t-test.

RESULTS

Trypsin activity: The trypsin activity on crude midgut homogenates of *R. ferrugineus* was standardized using different *p*H range (6-11), temperature regimes (37-50°C) and incubation time interval (20-40 min). The optimum conditions for trypsin activity with respect to the crude extract of *R. ferrugineus* are 50 mM Tris-HCl (*p*H 9.0) with incubation for 25 min at 40°C.

Among the six metal ions studied, 20 mM concentration of Na_2SO_4, $MgSO_4$, $CaCl_2$, $ZnSO_4$, were found to be stimulatory in that order and exhibited high residual specific activity of more than 50%. Sodium sulphate (Na_2SO_4) was found to be a cofactor for peptidase activity with respect to the crude extract of *R. ferrugineus* (Fig 1).

Results indicated the presence of trypsin activity in all stages of larval development of *R. ferrugineus*. Age related modulation of trypsin activity, protein concentration and specific activity was observed for crude midgut homogenate of *R. ferrugineus* grubs. Trypsin activity was found to be low at early instar (515.3 nanomole *p*NA / min / g), which attained a peak at mid instar (1043.0 nanomole *p*NA / min / g) and further reduced to lowest (288.9 nanomole *p*NA / min / g) at late instar indicating a peak activity at mid-instar of *R. ferrugineus* coinciding the active feeding stage of the insect (Table 1).

A progressive decline in the trypsin activity with increase in the concentration of the serine protease inhibitors *viz*., aprotinin, SBTI and PMSF was observed suggesting the presence of serine residue at active site of the enzyme. Results indicated that 50 µg of aprotinin, 30 µg of SBTI and 1700 µg of PMSF induced inhibitory effect to the tune of 77.4%, 63.1% and 55.9%, respectively, on trypsin activity of *R. ferrugineus*. Aprotinin was found to

Table 1. Trypsin activity on different instars of *R. ferrugineus*

Stage	Activity (nanomole *p*NA / min / g)	Protein (mg /g)	Specific activity (nanomole *p*NA / min / mg protein)
Early-instar	$515.3^{b} \pm 11.1$	$35.3^{b} \pm 2.3$	14.59
Mid-instar	$1043.0^{a} \pm 14.1$	$54.7^{a} \pm 2.9$	19.06
Late instar	$288.9^{c} \pm 7.6$	$27.1^{c} \pm 2.5$	10.66

In columns values followed by same alphabet(s) are not significantly different (P<0.05 DMRT)

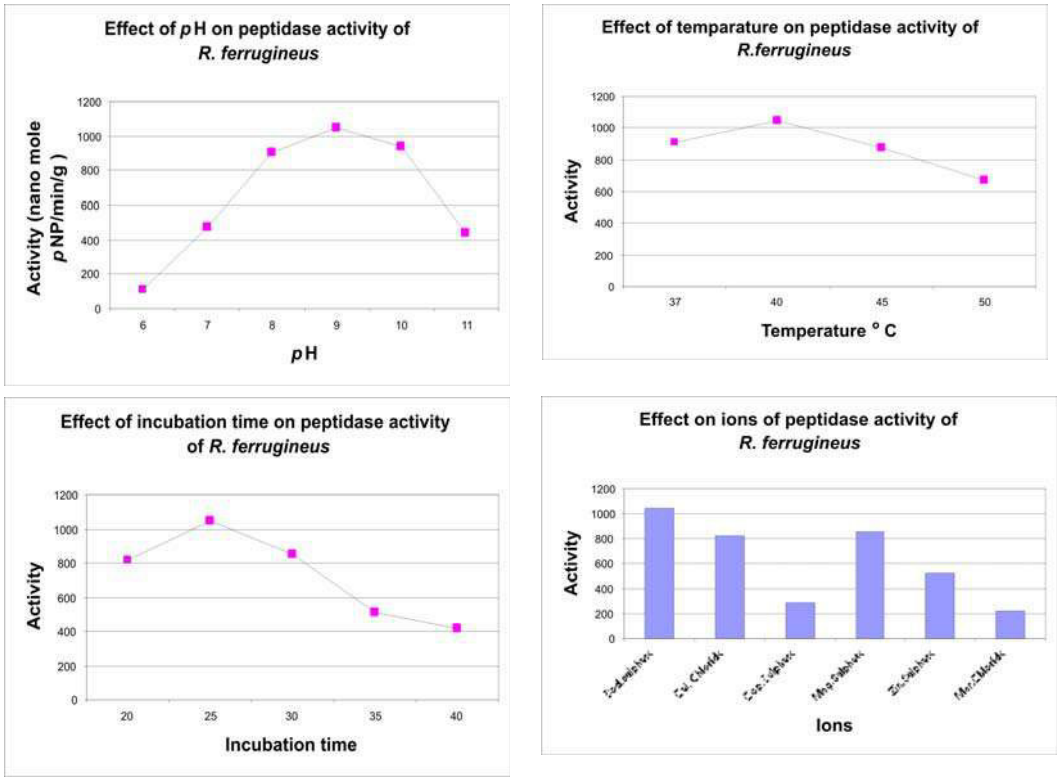

Fig. 1. Standardization of trypsin activity

be more inhibitory than SBTI whereas SBTI was found to be more inhibitory than PMSF for a given concentration of inhibitor on the peptidase activity of *R. ferrugineus* (Table 2). The inhibition pattern of trypsin activity on the crude mid gut homogenate of *R. ferrugineus* was found to be aprotinin > SBTI > PMSF in that order of magnitude.

Elastase-like chymotrypsin activity: The elastase-like chymotrypsin activity (SAAPL*p*NA-ase activity) on crude midgut homogenates of *R. ferrugineus* was standardized using different *p*H range (6-11), temperature regimes (37-55°C) and incubation time interval (20-50 min). Highest activity of elastase-like chymotrypsin in *R. ferrugineus* was recorded at 50 mM Tris-HCl (*p*H 8.0) with incubation for 25 min at 40°C. Among the six metal ions studied, 20 mM concentration of $CaCl_2$ and $MgSO_4$ were found to be stimulatory in that order and exhibited high residual specific activity of more than 60%. Calcium chloride ($CaCl_2$) was found to be a cofactor for elastase-like chymotrypsin activity with respect to the crude extract of *R. ferrugineus* (Fig. 2).

Elastase-like chymotrypsin was also found to be one of the dominant digestive proteinases of *R. ferrugineus* evincing maximum activity (882.6 nmole *p*NA rel/min/g) in mid-instar coinciding the active feeding stage of the insect. Elastase activity was found to be lowest at late-instar (244.3 nmole *p*NA rel/min/g) and comparatively higher at early-instar (354.7 nmole *p*NA rel/min/g) of *R. ferrugineus* (Table 3).

Serine proteinase inhibitors *viz*., aprotinin (50 µg), soybean trypsin inhibitor (30 µg) and phenyl methyl suphonyl fluoride (1700 µg) had a marginal reduction 32%, 14% and 11%, respectively in elastase-like chymotrypsin activity of *R. ferrugineus* suggesting the serine nature of the protease. Among the inhibitors evaluated, inhibition pattern of elastase

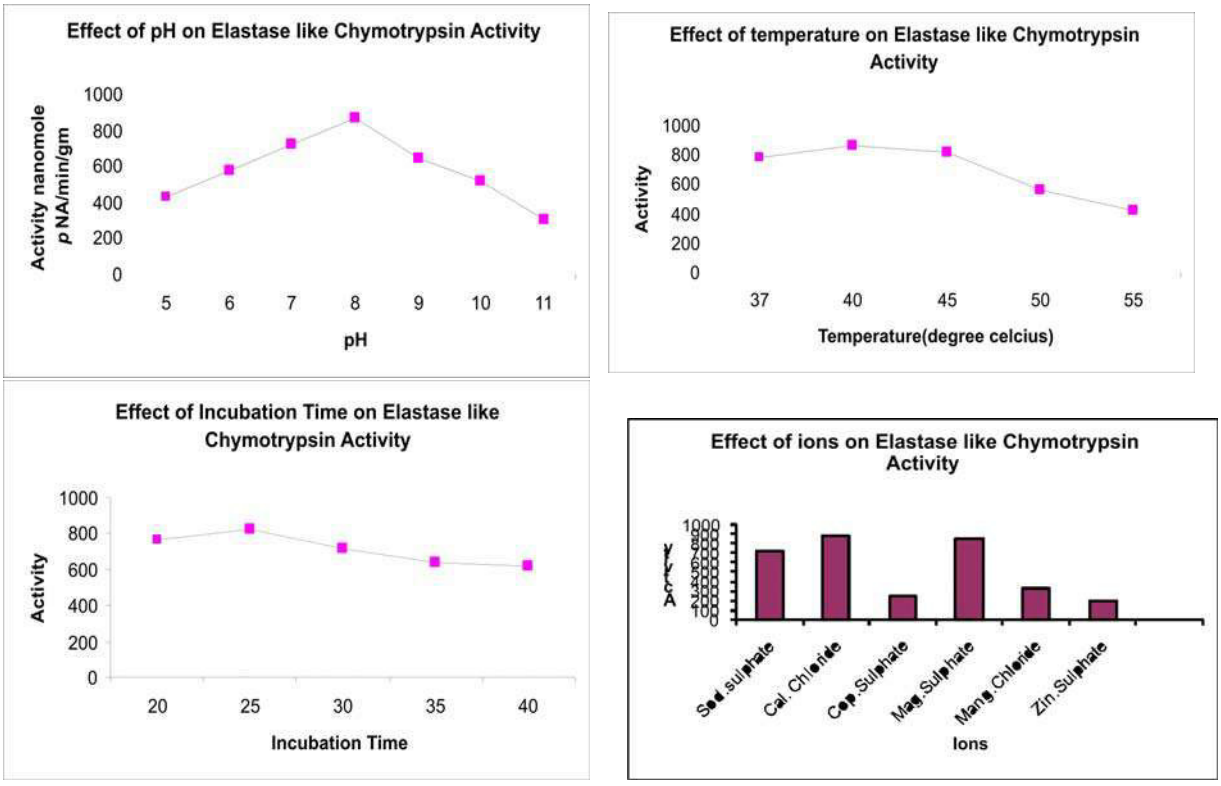

Fig. 2. Standardization of elastase-like chymotrypsin

Table 2. Influence of inhibitors on trypsin activity of *R. ferrugineus*

Aprotinin (?g)	Activity (nanomole *p*NA / min / g)	Soybean trypsin inhibitor (SBTI) (?g)	Activity (nanomole *p*NA / min / g)	Phenyl methyl sulphonyl fluoride (PMSF)	Activity (nanomole *p*NA / min / g) (?g)
0	$1044.8^{d} \pm 14.1$	0	$1044.8^{d} \pm 14.1$	0	$1044.8^{d} \pm 14.1$
10	$652.3^{c} \pm 5.9$	10	$497.3^{c} \pm 9.2$	170	$868.2^{c} \pm 5.9$
25	$295.4^{b} \pm 7.9$	25	$414.9^{b} \pm 4.8$	850	$770.9^{b} \pm 6.8$
50	$235.7^{a} \pm 4.3$	50	$385.6^{a} \pm 8.2$	1700	$459.9^{a} \pm 7.3$

In columns values followed by same alphabet(s) are not significantly different (P<0.05 DMRT)

Table 3. Chymotrypsin activity on different instars of *R. ferrugineus*

Stage	Activity (nanomole *p*NA / min / g)	Protein (mg /g)	Specific activity (nanomole *p*NA / min / mg protein)
Early-instar	$354.7^{b} \pm 13.3$	$36.6^{b} \pm 4.6$	9.7
Mid-instar	$882.6^{a} \pm 16.9$	$54.3^{a} \pm 2.7$	16.3
Late- instar	$244.3^{c} \pm 8.7$	$28.6^{c} \pm 3.2$	8.5

In columns values followed by same alphabet(s) are not significantly different (P<0.05 DMRT)

activity on the crude mid gut homogenate of *R. ferrugineus* was found to be aprotinin > soybean trypsin inhibitor > phenyl methyl suphonyl fluoride in that order of magnitude. The effect of various inhibitors on elastase-like chymotrypsin is presented in table 4.

***In vivo* feeding bioassay:** *In vivo* bioassay of aprotinin (250 mM) on coconut petiole painting method using larvae of *R. ferrugineus* revealed changes in the weight gain of the test insect. In a period of 120 h, the control insect attained 2.305 g weight whereas aprotinin-fed *R. ferrugineus* attained 1.939 g indicating weight loss of 18.9% due to incorporation of serine protease inhibitor.

DISCUSSION

Gut was considered as a vital target for insect control due to its importance in food digestion and nutrient absorption. Dietary protein digestion in insects is initiated by hydrolysis by endopeptidases, followed by carboxypeptidases and aminopeptidases. Endopeptidases degrade the proteins into small peptides, and aminopeptidases and carboxypeptidases further degrade the peptides into amino acids from the amino and carboxyl termini, respectively.

Gaining an insight into the proteolytic properties of the digestive enzymes of *R. ferrugineus* is critical for developing appropriate and effective pest management strategies through protease inhibitors. Results from these studies suggest that protein digestion in *R. ferrugineus* is primarily due to serine proteases that are sensitive to serine protease inhibitors tested. Digestion of food by serine proteases is the preferred mode in lepidopteran insects. Targeting these enzymes may be a good strategy for the development of effective bio-pesticides. Selective inhibition of digestive enzymes in insects induces production of detrimental effects on growth of larvae to prevent digestion and assimilation of nutrients to retard their development and cause their death. Gut homogenates of *R. ferrugineus* in this study displayed substantial enzyme activity only at alkaline *p*H with maximum values recorded at *p*H 9.0 for peptidase activity and *p*H 8.0 for elastase-like activity. This high value is not the same as the optimum *p*H reported for several species of coleopteran insects, which show a neutral (*p*H 7.0) or even slightly acidic (*p*H 5.0) optimum *p*H (Novillo *et al*., 1997). The results obtained in this study on crude gut homogenate of *R. ferrugineus* suggest a major involvement of alkaline proteases in protein digestion. The data strongly suggests the presence of serine proteinases in midgut extracts, confirming the occurrence of protein digestion in the insect.

The trypsin activity as well as chymotrypsin activity of *R. ferrugineus* progressively increased to reach a maximal activity at 40°C and thereafter due to inactivation of the enzyme and linearization of 3D configuration, there was a decline in activity attaining as low as only 59% at 50°C. The trypsin

Table 4. Influence of inhibitors on elastase-like chymotrypsin of *R. ferrugineus*

Aprotinin (µg)	Activity (nanomole *p*NA / min / g)	Soybean trypsin inhibitor (µg)	Activity (nanomole *p*NA / min / g)	Phenyl methyl sulphonyl fluoride (µg)	Activity (nanomole *p*NA / min / g)
0	$842.8^{d} \pm 18.1$	0	$849.8^{d} \pm 15.1$	0	$853.2^{d} \pm 17.1$
10	$830.1^{c} \pm 20.1$	10	$840.2^{c} \pm 17.2$	170	$843.4^{c} \pm 15.0$
25	$713.9^{b} \pm 17.5$	25	$798.2^{b} \pm 14.1$	850	$805.2^{b} \pm 12.1$
50	$573.4^{a} \pm 16.2$	50	$727.4^{a} \pm 15.9$	1700	$760.2^{a} \pm 15.1$

In columns values followed by same alphabet(s) are not significantly different (P<0.05 DMRT)

activity was strongly temperature dependent and was similar to that reported from several other lepidopteran larvae (Bernardi *et al*., 1991).

Some metal ions such as Na^+, Mg^{2+}, Ca^{2+} and Zn^{2+} enhanced BA*p*NA-ase / SAAPLpNA activities whereas others like Cu^{2+} and Hg^{2+} were inhibitory at 20 mM concentration. The specific activities of three ions *viz*., Na^+, Mg^{2+} and Ca^{2+} were found to be above 50 nanomole *p*NA released / min/ mg protein suggesting their possible role as cofactors for trypsin-lke proteases of *R. ferrugineus*. Accumulation of heavy metals due to excessive application of fertilizers and pesticide molecules could possibly alter the trypsin activity of *R. ferrugineus* leading to desensitization and adaptive behaviour. In crude mid gut homogenate of cardamom shoot and capsule borer, *C. punctiferalis* divalent ions such as Ca^{2+}, Mg^{2+}, Pb^{2+} and Co^{2+} exhibited stimulatory effects on peptidase activity, whereas Mn^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, and Hg^{2+} were inhibitory and the effect of other monovalent ions such as Na^+, K^+, Li^+, Rb^+ and Cs^+ were only marginal (Josephrajkumar *et al*., 2006). When monovalent ion such as Na^+ exhibited marginal effect on lepidopteran insect, *C. punctiferalis* it evinced highest peptidase activity on coleopteran insect, *R. ferrugineus* suggesting the uniqueness of coenzyme for each insect on peptidase activity. Predominance of coconut along sea shore may have contributed the sodium ion as a possible coenzyme for the peptidase activity of *R. ferrugineus* and the possible adaptation of this ion by insect. Absorption of sodium ion by the plant may therefore be well utilized by the insect for its peptidase activity.

During the active feeding stage of the grub the trypsin/chymotrypsin activity was found to be the highest indicating higher consumption of food as well as effective digestion of the food consumed. Food consumption by insects is highly correlated with trypsin/chymotrypsin activity in the gut. A significant amount of inhibitors may have to be ingested during early and active feeding stages of the test insect coinciding with the highest levels of activity of digestive proteinases. There has been a noticeable decrease in the specific activity at the late stage of larval development of *R. ferrugineus* may be coinciding the wandering stage prior to pupation. This decline may result from a greater degradation or a lower synthesis of digestive proteianses produced by a quantitative decrease of the feed intake when larvae is near of the next moult stage or approaching pupation. As the grubs approached pupation, lower levels of proteolytic activity are present in the insect guts, concomitant with decreased feeding activity. Elastase-like chymotrypsin activity was found to be lower than peptidase activity indicating the dominance of trypsin-like proteases in protein digestion of *R. ferrugineus*.

The study demonstrated that, *in vitro*, aprotinin, SBTI and PMSF were effective at retarding trypsin-like (BA*p*NA hydrolyzing) and elastase-like chymotrypsin (SAAPL*p*NA hydrolyzing) activity extracted from the digestive tract of *R. ferrugineus.* It was also found that the inhibition of both endopeptidases on the crude mid gut homogenate of *R. ferrugineus* was found to be aprotinin > SBTI > PMSF in that order of magnitude. As expected, aprotinin was particularly effective at inhibiting both the endopeptidases than the other two inhibitors studied. The results demonstrated a pronounced difference in the sensitiveness of enzyme activities to the inhibitor as the concentration of inhibitors varied for achieving the similar level of inhibition under *in vitro* condition. These results agree to those reported by Oliveira *et al*. (2005) who detected a higher sensitivity of the proteolytic activity of the partially purified fraction to benzamidine than to PMSF.

Besides inhibiting both the endodpeptidases studied, aprotinin significantly suppressed the growth of *R. ferrugineus* indicating effective indigestion of dietary proteins. This is also indicative for effective silencing of these insect specific serine proteinases for retarding growth of *R. ferrugineus*.

The work presents one of the first steps to more precise understanding of biochemical organization of digestive processes in *R. ferrugineus*. Future studies concerning *R. ferrugineus* with particular emphasis on enzyme compartmentalization, substrate specificity and substrate preference as

well as inhibition will deepen our understanding of the digestive processes within this polyphagous Curculionid beetle. Targeting and purification of the these enzymes may be good strategy for the development of effective bio-pesticides and developing transgenics.

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First report of six predatory mites (Acari: Phytoseiidae) from the central Indian state of Chhattisgarh

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ABSTRACT: Occurrence of of phytoseiid mites, *viz, Euseius delhiensis*, *Neoseiulus fallacis*, *Phytoseius kapuri*, *Typhlodromips syzygii* and two new species of *Amblyseiulella* and *Neoseiulella* is reported for the first time from the central Indian state of Chhattisgarh.

KEY WORDS: First report, phytoseiid mites, Chhattisgarh, India, vegetables

INTRODUCTION

Predatory mites have already gained acceptance among farmers worldwide as natural enemies that provide effective pest control in greenhouses and open fields. They are now commercially viable because of the range of crops on which they are used as a biocontrol option for phytophagous mites and small sucking insects like thrips and whiteflies. Predatory mites of Phytoseiidae are more valuable as they in general inhabit plants and offer sustainable control of pest mites. Till date 2,735 species of phytoseiids have been described from around the world, out of which, more than 210 species are found in India (Demite *et al*., 2014, 2016; Gupta and Karmakar, 2015). Phytoseiid fauna of most of the Indian states have already been explored (Gupta, 1986, 2003), except some regions like the central Indian state of Chhattisgarh, which is the tenth-largest state with predominant agricultural background. The present paper reports on the phytoseiid mites collected from the plains of Chhattisgarh.

MATERIALS AND METHODS

A roving survey was conducted for predatory mites in Purena of Raipur district (21°13'52"N; 81°42'44"E) and Abhanpur of Dhamtari district (21°03'57"N; 81°45'11"E) in Chhattisgarh. The samples collected from various vegetable crops, cotton and tapioca were examined under a stereozoom microscope (Nikon SMZ800) and mites were picked up with a fine camelhair brush moistened with 70–80% ethyl alcohol. In some cases, mites were washed from plant parts or shaken directly into jars filled with alcohol or water to which a surfactant had been added (Zacharda *et al*., 1998).

Mites were killed and fixed with freshly prepared 70–80% ethyl alcohol and mounted individually in Hoyer's medium on standard microscope slides. Slides were then kept on a hot plate at 40–45°C for 72 hours for clearing of specimens and drying of medium. Occasionally, slides were kept under a table lamp or in an oven for drying of medium for 2

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days. Dried slides were ringed with transparent nail polish. Cleared specimens were identified with the help of published keys and relevant literature (Gupta, 1986,& 2003) following the classification of Chant and McMurtry (2007). Measurements (in µm) were taken under a compound microscope (Leica DM1000) at 400× for comparisons with original descriptions. All the slides are available in the Mite Repository of ICAR–National Bureau of Agricultural Insect Resources, Bengaluru, India.

RESULTS AND DISCUSSION

***Euseius delhiensis* (Narayanan & Kaur)**

Typhlodromus (*Amblyseius*) *delhiensis* Narayanan & Kaur, 1960, *Proc. Indian Acad. Sci*., 51: 5–7.

Euseius delhiensis, Chant & Baker, 2007: 120.

Euseius delhiensis, Gupta & Karmakar, 2015: 59.

Measurements: Dorsal shield smooth, 328 long, 213 wide, with 17 pairs of setae; j_1–30–33, j_3–35–40, j_4–13–15, j_5–15–18, j_6–23–25, J_2–23–25, J_5–4–5, z_2–27–30, z_4–40–43, z_5–13–15, Z_4–25–28, Z_5–60–63, s_4–55–58, S_2–25–28, S_4–23–25, S_5–28–33, r_3–18–20, R_1–13–15; sternal shield 73, longer than broad, with three pairs of setae; genital shield 88 wide with a pair of setae; ventrianal shield 95 long, 70 wide, with a pair of crescent-shaped preanal pores and three pairs of preanal setae arranged in two transverse curved rows; a pair of metapodal plates present, primary one 25, secondary one smaller; fixed digit of chelicera with three apical teeth, movable digit with one tooth; macrosetae on leg IV: genu–53–55, tibia–40–45, basitarsus–73–75.

Leg chaetotactic formulae: Genu II 2, 2/0, 2/0, 1; genu III 1, 2/1, 2/0, 1; tibia II 1, 1/1, 2/1, 1; tibia III 1, 1/1, 2/1, 1.

Remarks: This was earlier unknown from Chhattisgarh.

Distribution in India: Delhi, Kerala, Odisha, Punjab, Tamil Nadu, Uttar Pradesh, West Bengal and Chhattisgarh (new report).

***Neoseiulus fallacis* (Garman)**

Iphidulus fallacis Garman, 1948, *Bull. Conn. Agr. Expt. Sta*., 520: 13.

Neoseiulus fallacis, Chant & McMurtry, 2007: 24.

Neoseiulus fallacis, Gupta & Karmakar, 2015: 53.

Measurements: Dorsal shield–378 long, 193 wide; j_1–30, j_3–50, j_4–28, j_5–38, j_6–43, J_2–53, J_5–13, z_2–45, z_4–50, z_5–28, Z_1–48, Z_4–66, Z_5–75, s_4–60, S_2–58, S_4–55, S_5–45, r_3–50, R_1–48; sternal shield 80 long, 82 wide; genital shield 72 long; ventrianal shield 128 long, 103 wide, with three pairs of preanal setae and a pair of crescent-shaped preanal pores; metasternal plate 15 long; two pairs of metapodal plates present, primary one 30 long; macrosetae on leg IV: genu–18, tibia–30, basitarsus–60; fixed digit of chelicera multidentate with *pilus dentilis* and movable digit with one tooth.

Leg chaetotactic formulae: Genu II 2, 2/0, 2/0, 1; genu III 1, 1/1, 2/1, 1; tibia II 1, 2/1, 1/1, 1; tibia III 1, 1/1, 2/1, 1.

Remarks: The measurements taken in the present study are similar to those given by Gupta (2003). This species was unknown from Chhattisgarh.

Distribution in India: Andaman and Nicobar Islands, Arunachal Pradesh, Assam, Bihar, Haryana, Himachal Pradesh, Madhya Pradesh, Meghalaya, Punjab, Tamil Nadu, Tripura, West Bengal and Chhattisgarh (new report).

***Phytoseius kapuri* Gupta**

Phytoseius (*Phytoseius*) *kapuri* Gupta, 1969, *Israel J. agric. Res*., 19(3): 115–117.

Phytoseius kapuri, Chant & McMurtry, 2007: 129.

Phytoseius kapuri, Gupta & Karmakar, 2015: 60.

Measurements: Dorsal shield 265 long, 135 wide; j_1–25–28, j_3–70–73, j_4–4–5, j_5–4–5, j_6–4–5, J_2–10–13, J_5–4–5, z_2–10–13, z_4–8–10, z_5–3–5, Z_4–75–78, Z_5–80–85, s4–105–110, s6–90–93, r_3–45–48, R_1–15–20; sternal shield wider (85) than long (80) with three pairs of sternal setae; genital shield 75 wide, 48 long; ventrianal shield 88 long, 56 wide, with three pairs of preanal setae and length of JV_4–53–

55; spermatheca bell-shaped; macrosetae on leg IV: genu–28, tibia–33, basitarsus–28.

Leg chaetotactic formulae: Genu II 2, 2/0, 2/0, 1; genu III 1, 2/0, 2/0, 1; tibia II 1, 1/1, 2/1, 1; tibia III 1, 1/1, 2/1, 1.

Remarks: Most measurements of the specimens collected are similar to those reported by Gupta (2003) from different states of India. This species was unknown from Chhattisgarh.

Distribution in India: Andaman and Nicobar Islands, Arunachal Pradesh, Assam, Bihar, Gujarat, Jammu and Kashmir, Kerala, Madhya Pradesh, Meghalaya, Odisha, Puducherry, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal and Chhattisgarh (new report).

***Typhlodromips syzygii* Gupta**

Amblyseius syzygii Gupta, *Int. J. Acarol*., 1(2): 44–45.

Typhlodromips syzygii, Chant & Baker, 2007: 63.

Typhlodromips syzygii, Gupta & Karmakar, 2015: 55.

Measurements: Dorsal shield 338 long, 230 wide; j_1–15, j_3–23, j_4–10, j_5–10, j_6–13, J_2–13, J_5–8, z_2–13, z_4–13, z_5–10, Z_1–13, Z_4–38, Z_5–68, s_4–25, S_2–13, S_4–8, S_5–8, r_3–15, R_1–13; sternal shield 68 long, 85 wide, with three pairs of setae; metasternal plate with setae conspicuous, 13 long, 8 wide; genital shield 83 wide; ventrianal shield vase-shaped, with lateral margins concave, 113 long, 73 wide, with three pairs of preanal setae and a pair of crescent-shaped preanal pores, four pairs of setae present around ventrianal shield, JV_5–30–35 long; a pair of metapodal plates present, primary one 20 long, longer than secondary one; fixed digit of chelicera with three teeth anterior to *pilus dentilis*, three teeth posterior to it, movable digit also with three teeth; macrosetae on genu I–23, genu II–25, genu III–30, genu IV–38, tibia IV–33, basitarsus IV–40.

Leg chaetotactic formulae: Genu II 2, 2/0, 2/0, 1; genu III 1, 2/1, 2/0, 1; tibia II 1, 1/1, 2/1, 1; tibia III 1, 1/1, 2/1, 1.

Remarks: Earlier this species was unknown from Chhattisgarh.

Distribution in India: Bihar, Odisha, Tripura, Uttar Pradesh, West Bengal and Chhattisgarh (new record).

***Amblyseiulella* sp.**

Measurements: Dorsal shield 300 long and 158 wide; j_1–36–38, j_3–85–90, z_2–20–25, z_4–30–38, Z_4–68–72, Z_5–90–95, s_4–125–128, r_3–45–50, R_1–20–22; sternal shield weakly sclerotized, 78–83 long, 70–73 wide with three pairs of sternal setae; metasternal plates 12–13; genital shield wider (98) than ventrianal shield, with a pair of setae; ventrianal shield smooth, 90 long, 62 wide, with three pairs of preanal setae, JV_5–62–65; a pair of metapodal plates present, primary one 32–35 long: fixed digit multidentate, movable digit with 3 teeth; macrosetae on leg IV: genu–23–25, tibia–33–35, basitarsus–41–43, distitarsus–33–35; genu I and II also having one knobbed macroseta each.

Leg chaetotactic formulae: Genu II 2, 2/0, 2/0, 1; genu III 1, 2/1, 2/0, 1; tibia II 1, 1/1, 2/1, 1; tibia III 1, 1/1, 2/1, 1.

Remarks: Earlier no species of this genus was known from Chhattisgarh. This does not tally with any of the known species of *Amblyseiulella* and therefore is likely to be new to be described elsewhere.

Distribution in India: Arunachal Pradesh and Chhattisgarh (new report).

***Neoseiulella* sp.**

Measurements: Dorsal shield lightly sclerotised and reticulated with 375 long, 245 wide; j_1–28, j_3–40, j_4–35, j_5–33, j_6–38, J_2–50, J_5–8, z_2–25, z_4–45, z_5–33, Z_1–55, Z_4–53, Z_5–65, s_4–50, S_2–60, S_4–55, S_5–10, r_3–43, R_1–45; sternal shield longer 100 than wide 90, with three pairs of sternal setae; metasternal plate 13 long, 7 wide with a seta; genital shield 83 long, 90 wide; ventrianal shield bullet-shaped, 133 long, 88 wide, along with three pairs of preanal setae; two pairs of metapodal plates present, primary one 48 long, secondary one small;

macrosetae on leg IV: genu–20, tibia–27, basitarsus–45.

Leg chaetotactic formulae: Genu II 2, 2/1, 2/0, 1; genu III 1, 2/1, 2/0, 0.

Remarks: This species is close to *Neoseiulella transitans* (Gupta) but significantly differs in the dorsal chaetotaxy. This species is under further investigation and will be described as new only after its confirmation of novelty.

Distribution in India: New Delhi, Jammu and Kashmir, West Bengal and Chhattisgarh (new report).

Most predatory mites in India remain unreported and underexploited. The plant mites of Chhattisgarh in general and predatory phytoseiid mites in particular are almost totally unexplored though this state is one of the largest in India and is rich with biodiversity as well as with agricultural products. For example, there has only been one report from Chhattisgarh of a predatory mite (*Euseius* sp.) observed during insecticide trials (Sarathi, 2011) on *Jatropha curcas* L. in Raipur. In the present limited study, *Euseius delhiensis* and *Phytoseius kapuri* were abundantly found on cotton (*Gossypium hirsutum* L.) and eggplant (*Solanum melongena* L.), respectively. The occurrence of other mites on vegetables was casual in nature, the presence of *N. fallacis* on tapioca (*Manihot esculenta* Crantz) in Abhanpur and *T. syzygii* on cluster bean [*Cyamopsis tetragonobola* (L.) Taub.] in Purena was noteworthy. The undescribed *Amblyseiulella* and *Neoseiulella* species were found on pumpkin (*Cucurbita pepo* L.) and cluster bean, respectively. The present study highlights the abundance of mites on vegetable crops which need to be explored and documented to enrich the mite faunal wealth of Chhattisgarh. This study has given us only an indication of the large diversity of unidentified predatory mites in the central parts of India. It was a good indication of biological control, in which predatory mites found abundantly on vegetables, one of the most vulnerable crops for mite pests. Interestingly, *P. kapuri* and *N. fallacis* were earlier reported (Gupta, 1986, 2003) from undivided Madhya Pradesh, the parent state of Chhattisgarh.

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Biology and morphometrics of root mealybug *Formicococcus polysperes* Williams (Hemiptera: Pseudococcidae) infesting black pepper (*Piper nigrum* Linnaeus)

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ABSTRACT: Studies on the biology of *Formicococcus polysperes* Williams infesting roots of black pepper (*Piper nigrum* Linnaeus) revealed females reproduced ovoviviparously and the reproductive period including pre larviposition, larviposition and postlarviposition periods lasted for an average of 23.65 ± 2.01, 9.6 ± 3.34 and 4.15± 0.93 days respectively. Gravid females gave birth to 136.15 ± 74.93 crawlers. Development period of females included three nymphal instars whereas males had two nymphal instars, a pre pupal and pupal stages. Duration of first two nymphal instars, third female nymphal instar, pre-pupal and pupal stages 8.4 ± 2.46, 6.35 ± 1.95, 8.4 ± 1.87, 1.4 ± 0.50 and 7.15 ± 0.88 days respectively. Adult males were short lived (1.8 ± 0.52 days) and adult females lived for 37.4 ± 3.10 days. Total life cycle of males was shorter (23.7 ± 3.01days) than that of females (60.55 ± 5.36 days). The sex ratio was 1.00:2.71 (male: female). The morphometric data of all stages are presented.

KEYWORDS: Root Mealybug, *Formicococcus polysperes,* life cycle, morphometrics, *Piper nigrum*

INTRODUCTION

Mealybugs are important pests of black pepper (Koya *et al.,* 1996) and its infestation on roots of black pepper were reported from different districts of Kerala. Higher infestation was reported in Wayanad (8.0 to 21.1 %) and lower in Idukki (0 to 3%). Stray infestation of the pest was observed in Kozhikode and Kannur districts (Devasahayam *et al.,* 2010). *Planococcus* sp., *P. citri* (Risso), *P. lilacinus* (Ckll.), *Dysmicoccus brevipes* (Ckll.) and *Ferrisia virgata* (Ckll.) were reported to be infesting roots and basal portions of stems (under the soil) of black pepper vines. Colonies of these root mealybugs were distributed on the main, secondary and tertiary roots and basal region of stems on rooted cuttings in the nursery and also on the vines of all age groups in the field.

Severe infestation resulted in defoliation, yellowing and wilting of leaves and lateral branches and also mortality of vines (Devasahayam *et al.,* 2010). Another hypogeal mealybug species, *Formicococcus polysperes* Williams (Homoptera: Pseudococcidae) which is known to infest root region of crops of different families was also observed on the roots of black pepper in Kerala. Williams (2004) described this species from roots of *Macaranga triloba* (Thunberg) Müller Argoviensis from Malaysia and reported its

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distribution and host plants. It was reported on roots of *Macaranga triloba, M. conifer* (Reichen-bach & Zollinger) and *Sapium buccatum* Roxburgh (Euphorbiaceae) from Malaysia, *Zingiber officinale* Roscoe (Zingiberaceae), *Cocos nucifera* L. And *Rhapis excels* (Thunberg) Henry (Aracaceae) from Philippines, *Z. officinale* from Thailand and *Lansium domesticum* Corrêa from Vietnam. In India, it has been reported on roots of *Piper nigrum* L. (Kerala), *P. betle* L. (Madhya Pradesh, Uttar Pradesh, and West Bengal), *Areca catechu* L. (Uttar Pradesh) and on pods of *Arachis hypogaea* L. (Orissa) (Williams, 2004). Detailed biology and morphometrics of *F. polysperes* was undertaken for the first time.

MATERIALS AND METHODS

Studies were undertaken in the laboratory of department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University. The temperature during the study period (February to April 2015) ranged from 29.4°C and 31.7°C and relative humidity was 57 - 82 per cent.

Identification of mealybug species: Root mealybugs were collected from pepper gardens of Wayanad and Idukki districts of Kerala. The collected samples were preserved separately in 70 % ethyl alcohol and sent to National Bureau of Agricultural Insect Resources, Bengaluru for identification.

Laboratory rearing of mealybugs: Mature pumpkin (*Cucurbita moschata* Duch.) fruits with abundant grooves were used as substrate for mass rearing of mealybugs. Fresh pumpkin fruits were washed thoroughly with water, disinfected with 0.1% carbendazim and air dried. Such pumpkins were tied with twine along the grooves for easy establishment of the mealybugs and kept in aluminium netted rearing cages kept at temperature of 27-28°C. Ant pans were maintained to prevent the entry of ants into the cage. The adult mealybugs collected from pepper gardens were released at the stalk region of pumpkin and covered with a steel bowl for 7 days to provide darkness and to restrict the movement of mealybugs so that they settled easily. The bowl was removed after the mealybugs settled on the pumpkin.

Biology: Cut portions of pepper cuttings (from runner shoots)with at least one leaf node and aerial root was selected as the substrate for the study of biology. Eggs were not observed during the study and hence, one day old first instar nymphs (crawlers) were released near tothe leaf node of pepper cuttings using camel hair brush. Nymphs used for the study were taken from single female. The pepper cuttings were kept in Petriplates lined with a layer of wet absorbent cotton and observed daily for recording the number and duration of nymphal instars. Moulting was confirmed by examining the presence of exuviae under stereoscopic microscope and removed after each moult. Twenty replications were maintained. Adult females were kept separately on pepper cuttings to observe pre-larviposition, larviposition and post larviposition periods. Twenty replications were maintained.Adult females were observed daily to record number of crawlers produced. Nymphs produced were removed daily with soft camel hair brush to avoid repeated counting. Nymphs from each female were reared separately and observed till the males and females can be distinguished for determination of sex ratio. The nymphs forming cocoons were separated as males. Adult longevity of females and males were observed separately.

Morphometry: Morphometric data of all stages were measured using stereo zoom microscope (Lieca®) with image analyzer facility. Body length and width of 20 individuals of all stages were measured to determine body size. Length was measured dorso-medially from the head to the tip of the abdomen. Width was measured at the widest part of body.

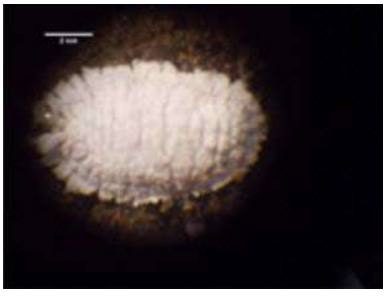
RESULTS AND DISCUSSION

Pre-larviposition, larviposition and post larviposition periods lasted for an average of 23.65 ± 2.01, 9.6 ± 3.34 and 4.15± 0.93 days, respectively. Adult females of *F. Polysperes* gave birth to first instar nymphs (crawlers) ovoviviparously, into a cotton like wax threads secreted from the posterior part

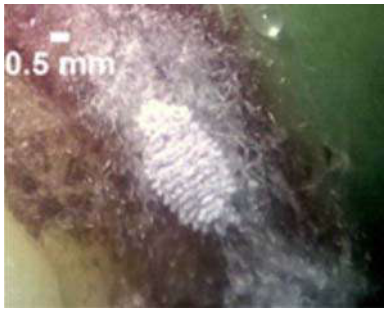
a. First instar nymph

b. Second instar nymph

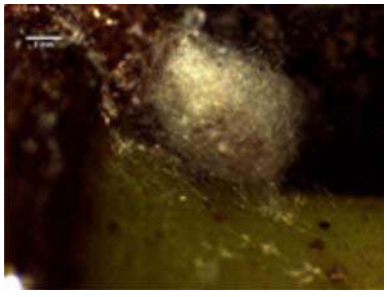
c. Third instar female nymph



d. Adult female



e. Pre pupa



f. Pupa

g. Male nymph inside the cocoon

h. Adult male

Fig. 1. Life cycle of *Formicococcus polysperes*

of the body. Adult female producedan average of 136.15 ± 74.93 crawlers and sex ratio was 1: 2.71 (male: female). Males and females of *F. polysperes* exhibited variation in its development stages. The female had three nymphal instars while the male had two (Table 1).

First instar: Freshly delivered first instar nymphs were oval, light pink with three pairs of legs and a pair of filiform antennae (Fig. 1a). Body colour changed from pink to pale white within a day after larviposition. Length of first instar nymphs was 0.89 ± 0.09 mm whereas width was 0.51 ± 0.06 mm. Duration of first nymphal instar lasted for 8.4 ± 2.46 days.

Second instar: Both first and second instar nymphs were similar in appearance and morphological characteristics except in body size (Fig. 1b). Wax coating was absent on body and secreted after about 24 hours of moult. Length and width of second instar nymph were 0.89 ± 0.09 mm and 0.51 ± 0.07 mm, respectively. Duration of second instar lasted for 6.35 ± 1.95 days.

Third instar: Males and females could distinguish from third instar onwards. After second instar,fine silken waxy threadswere formed by males which was absent in females. Hence, from this stage onwards, the observations were taken separately for males and females.

Table 1. Biology and morphometrics of *Formicococcu spolysperes*

Stage	n	Duration (Days)		Length (mm)		Width (mm)	
		Range	Mean	Range	Mean	Range	Mean
Development period							
First instar nymph	20	6-14	8.4 ± 2.46	0.64 - 0.98	0.89 ± 0.09	0.35 - 0.59	0.51 ± 0.06
Second instar nymph	20	5-10	6.00 ± 1.21	1.02 - 1.69	1.39 ± 0.25	0.56 - 0.99	0.80 ± 0.14
Third instar female nymph	20	6-13	8.4 ± 1.87	1.71 - 2.47	2.10 ± 0.26	0.91 - 1.82	1.25 ± 0.22
Pre-pupa	20	1-2	1.4 ± 0.50	1.01 - 1.62	1.29 ± 0.21	0.55 – 0.86	0.65 ± 0.11
Pupa	20	6 - 9	7.15 ± 0.88	1.56 - 2.41	2.03 ± 0.27	0.49 - 0.92	0.82 ± 0.13
Male	20	1-3	1.8 ± 0.52	0.78 - 1.57	1.13 ± 0.26	0.24 - 0.46	0.33 ± 0.06
Female	20	30 - 41	37.4 ± 3.10	2.1 - 3.25	2.65 ± 0.32	1.3 - 1.94	1.56 ± 0.24
Prelarviposition	20	21-29	23.65 ± 2.01	-	-	-	-
Larviposition	20	4-15	9.6 ± 3.34	-	-	-	-
Post larviposition	20	3-6	4.15± 0.93	-	-	-	-
Larviposition	20	76-357	136.15 ± 4.93	-	-	-	-
Total lifecycle							
Male	20	20 - 31	23.7 ± 3.01	-	-	-	-
Female	20	49 - 70	60.55 ± 5.36	-	-	-	-

*n: No. of observations/ replications

Third instar female nymph: Waxy filaments along the body margin were prominently visible from third instar onwards and nymphswere similar to adult females except in body size (Fig. 1c). Length of third instar female nymph was 2.10 ± 0.26 mm whereas width was 1.25 ± 0.22 mm. Duration of third instar was 8.4 ± 1.87.

Pre-pupa: This stage was identified by the presence of fine waxy threads which was later formed into a cocoon(Fig. 1e)**.** Duration of this instar lasted for an average of 1.4 ± 0.50 days. Morphometics of pre-pupal instar was similar to that of second instar with length and width of 1.29 ± 0.21 mm and 0.65 ± 0.11 mm, respectively.

Pupa: Male nymphs secreted waxy threads to form cocoon which covers the entire body. Cocoon was cylindrical and exuviae was present outside with which second moulting was confirmed (Fig. 1f). The male nymph inside the cocoon was dark pink in colour, slender, with a pair of 10 segmented antennae which was directed backwards along body margin and with wing pads. Waxy coating was absent (Fig. 1g). Duration of pupal instar lasted for an average of 7.15 ± 0.88 days. Length and width of male pupa was 2.03 ± 0.27 mm and 0.82 ± 0.13 mm, respectively.

Adult: Females of were apterous, soft bodied, oval and pink. Body segmentation was visible with powdery wax secretion. Waxy filaments surrounding the body margin are short and thick (Fig. 1d). The morphometric measurements of adult female was 2.65 ± 0.32 mm length and 1.56 ± 0.24 mm width. Males were slender, delicate, elongated and reddish brown with a pair of well developed, pale white and opaque wings, a pair of long waxy caudal filaments. A pair of long, 10 segmented antennae was also present which was characteristic of male (Fig. 1h). Male measured 1.13 ± 0.26 mm in length and 0.33 ± 0.06 mm width. Males were short lived when compared to females. Longevity of males was 1.8 ± 0.52 days and that of females

was 37.4 ± 3.10 days. Males had shorter life cycle than that of females which was lasted for an average of 23.7 ± 3.01. Total life cycle of females wasan average of 60.55 ± 5.36.

Bio-ecology, natural enemies and control measures of *F. polysperes* are not reported so far. The only information available on the pest is about its host and distribution by Williams (2004) and its infestation (48.3 %) on ginger in Meghalaya by Firake *et al.* (2015). The present study on the biology and morphometrics of *F. polysperes*is provides basic information for the first time which would help to investigate further applied aspects of the pest. Trapeznikova and Gavrilov (2008) supports the ovoviviparous mode of reproduction in *F. polysperes*in which eggs hatch inside the reproductive system of females and deliver the hatched out young ones. Another genus of *Formicococcus, F. njalensis* (*Pseudococcus njalensis*) also reproduced ovoviviparously with low fecundity varying from 6 to 90 (Strickland, 1951). Life cycle of female *F. polysperes*is was similar to that of *F. njalensis* in with three nymphal instars were reported with average duration of 7, 5 and 7 days respectively for first, second and third nymphal instar. The pre oviposition period recorded in *F. njalensis* was 23 days and is similar to *F. polysperes* in the present study (Strickland, 1951).

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Enhancing *in vivo* foraging activities of *Trichogramma chilonis* Ishii and *Chrysoperla zastrowi sillemi* (Esben-Peterson) on eggs of *Corcyra cephalonica* Stainton through kairomonic activity of *Helicoverpa armigera* (Hubner)

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ABSTRACT: Bioassay of hexane extracts (1000 ppm) of male and female whole body, frass and larval wash of host *Helicoverpa armigera* (Hubner) against *Trichogramma chilonis* Ishii and *Chrysoperla zastrowi sillemi* (Esben-Peterson) revealed their kairomonal activities under in vitro condition. Treating irradiated eggs of *Corcyra cephalonica* Stainton with hexane extract of adult female whole body of *H. armigera* (1000 ppm) recorded the parasitization of 17.34 per cent by *T. chilonis* on third day after inoculation which increased from 50.64 to 64.28 per cent on fifth and seventh day after inoculation and they were 7.94, 21.76 and 32.58 per cent when the eggs were treated with hexane alone on third, fifth and seventh days after inoculation, respectively. Maximum emergence (48.16%) was observed with H. *armigera* female whole body extract followed by male whole body extract (39.33%). The highest predation by *C. zastrowi sillemi* on hexane extract of *H. armigera* female whole body treated eggs of *C. cephalonica* was recorded (61.13%) whereas it was 37.85 per cent in hexane treated eggs.

KEY WORDS: *Chrysoperla zastrowi sillemi*, *Corcyra cephalonica*, *Helicoverpa armigera,* kairomone, *Trichogramma chilonis, foraging activities*

INTRODUCTION

Natural enemies detect chemical cues that are emanating from the host insects which help in their host location. Number of chemicals released from hosts, host secretions, hosts by-products and associated organisms influence the behaviour of natural enemies. Foraging female insect parasitoids use these chemical cues extensively to locate, identify and exploit their host in different eco-system (Penaflor *et al*., 2012; Parthiban *et al*., 2015). Many types of stimuli influence the habit location and host selection behaviour of parasitoids and predators among which the semiochemicals play a major role (Kumar and Ambrose, 2014; Joachim and Weisser, 2015). Similarly, host insects also contain saturated long chain hydrocarbons on their body surfaces. The surface hydrocarbon composition is observed to be species specific in insects. These saturated long chain hydrocarbons that are present on the surface of host plants and host insects have been reported to elicit synomonal and kairomonal responses in *Trichogramma* spp. The behavioural responses of *Trichogramma* spp. to synthetic

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hydrocarbons has been reported by Grenier *et al*. (1993). The host insects contain characteristic hydrocarbons, fatty acids and proteins present in their body or byproduct, which act as stimulants or arrestants to the parasitoids to intensify their search in the near vicinity of the host.

Saturated long chain hydrocarbons present on the body surface of *Spodoptera litura* (Fab.) and *Earias vitella* (Fab.) moths have been reported to elicit kairomonal response in Trichogramma spp. (Maruthadurai *et al*., 2011). In order to evaluate the role of kairomones released by host insect on parasitism and predation by *T. chilonis* and *C. zastrowi sillemi* laboratory bioassay were conducted with the hexane extracts (1000 ppm) of male and female whole body, frass and larval wash of host *H. armigera* to explain the kairomonal interaction between the parasitoid, predator and the host.

MATERIALS AND METHODS

Laboratory studies were carried out at Bio-control laboratory, Agricultural College and Research Institute, Madurai during 2014 to 2016 to study the kairomonal effect of *H. armigera* to natural enemies. Larvae of *H. armigera* collected from field were reared separately in multi-cavity tray containing chickpea flour based semi-synthetic diet. Old diet was replaced with fresh ones in alternate days. Pre-pupae were collected in vermiculite for pupation. Pupae collected from culture were placed in adult emergence cage measuring 30 x 30 x 30 cm. Five pairs of newly emerged adults were transferred to plastic buckets of seven litre capacity maintaining the sex ratio of 1:1 for oviposition. Adults were fed with 10 per cent sugar solution enriched with multivitamin drops. The mouth of the bucket was covered with sterile muslin cloth which served as oviposition substrate. The buckets were kept in a dark place at 25° C with 75% RH. Muslin cloth along with eggs was collected from third-day onwards and used for experiment (Parthiban *et al*., 2014).

C. cephalonica was reared in the laboratory as per the protocol suggested by Navarajanpaul (1973). The egg parasitoid, *T. chilonis* was mass cultured on the eggs of *C. cephalonica* as per the method described by Prabhu (1991). Mass rearing of *C. zastrowi sillemi* was carried out with *C. cephalonica* eggs as feed, as per the method described by Swamiappan (1996).

The whole bodywash from adult male, female, larvae and frass of moth of *H. armigera* was prepared as per the method described by Ananthakrishnan *et al*. (1991). Freshly emerged, healthy, 0-24 hrs old moths of male and female were collected and kept in a deep freezer (REMI model) at –20°C for 15 min for immobilization. Subsequently, 10 g of moths, third instar larvae and larval frass were weighed and soaked in 100 ml of distilled hexane (HPLC grade) for 24 hrs and shaked in water bath (Genuine model) at 28°C for two hours followed with 20 minutes at 50°C.These were filtered through Whatman No.1 filter paper. The hexane fraction was subsequently concentrated by vacuum evaporation at 40° C (LARK model). The extracts were stored at –20°C in deep freezer till further use for bioassay studies. A concentration of 0.1% (1000 ppm) of the extract of host insect was prepared after dilution with hexane and used throughout the experiment.

Bioassay studies of whole body wash, larval and frass exuding kairomones of host insects were carried out at 26 ± 2°C and 75 ± 5% R.H. and photoperiod 16:8 h scoto/photo regime. The procedure adopted was similar to the one described by Lewis et al. (1975). Clean, healthy, 0-24 hrs old eggs of *C. cephalonica* sterilized under UV light for 45 minutes were washed twice in hexane to remove any trace of scales or kairomones present on the surface of eggs. These eggs were pasted with pure white gum on dull coloured cardboard, measuring 7 x 2 cm at the rate of average of 0.05 cc eggs per piece (egg card). Kairomone extracts (1000 ppm) of *H. armigera* (male moths, female moths, larvae and frass extracts) used to treat the hexane washed eggs, separately and shade dried. Each egg card was considered as one replication and each treatment was replicated eight times. Control was maintained with hexane alone.

Egg card taken in a glass tube (7.5 x 2.5 cm) was introduced with freshly emerged *T. chilonis* adults (6:1). Per cent parasitization was observed on 3rd, 5th and 7th days after introduction. Similarly, five second instar of *C. zastrowi sillemi* was released in a vial with hexane washed *C. cephalonica* eggs (700-750) and per cent predation was calculated 24 hr after release (Murali Baskaran, 2013).

Data obtained from the bioassay of body washes of host insects were subjected to ANOVA (Analysis of Variance). Before analysis, data on per cent parasitism were transferred by arcsine transformation. In order to know the interaction between treatments, data from laboratory bioassay were subjected to factorial CRD (Completely Randomized Design) analysis and the means obtained were separated by LSD (Least Significant Difference) (Gomez and Gomez, 1984).

RESULTS

The results on parasitism corroborated that the highest mean per cent parasitism (44.09%) by *T. chilonis* was recorded in hexane extract of female whole body wash of *H. armigera* (1000 ppm) followed by 36.16 percentage in male whole body wash. Among the host insect washes larval and frass extract recorded the lowest mean percentage parasitism of 26.68 and 23.26, respectively, whereas the control (hexane) recorded the least mean parasitism (20.76). When the interaction between the different washes were analysed, it was found that the female body wash of *H. armigera* recorded the highest mean parasitization level of *T. chilonis* on eggs of *C. cephalonica*, recording 17.34, 50.64 and 64.28 per cent on 3rd, 5th and 7th day after introduction of parasitoids, respectively which was significantly different from hexane extract of male whole body (11.52, 41.05 and 55.92%), larval extract (8.89, 31.22 and 39.94%) and frass extract (9.12, 23.83 and 36.83%) while it was 7.94, 21.76 and 32.58 per cent parasitization in hexane alone treated eggs (Table 1).

Similarly, the highest mean per cent emergence (48.16%) was recorded in female body wash of *H. armigera* followed by male body wash (39.33%) (Table 2). The lowest mean emergence was recorded in frass extract (25.94%) among the different washes followed by larval extract (27.83%) and the lowest mean per cent emergence was recorded in control (22.15%).

Predatory activity of *C. zastrowi sillemi* was enhanced from 37.85 per cent (hexane treated eggs of *C. cephalonica*) to 61.13 per cent (Table 3), 24 hr after treatment when treated with hexane extract

Table1. Parasitism by *Trichogramma chilonis* on eggs of *Corcyra cephalonica*, as influenced by hexane extracts of *Helicoverpa armigera*

Insect samples	% parasitization by *T. chilonis* after*			Mean
	3rd day	5th day	7th day	
Male whole body	11.52(19.84)[b]	41.05(39.85)[b]	55.92(48.40)[b]	36.16(36.97)[b]
Female whole body	17.34(24.61)[a]	50.64(45.37)[a]	64.28(53.30)[a]	44.09(41.67)[a]
Frass extract	9.12(17.57)[c]	23.83(29.22)[d]	36.83(37.36)[d]	23.26(28.83)[d]
Larval extract	8.89(17.34)[c]	31.22(33.97)[c]	39.94(39.20)[c]	26.68(31.10)[c]
Control (Hexane)	7.94(16.36)[d]	21.76(27.81)[e]	32.58(34.81)[e]	20.76(27.11)[e]
SEd	0.3845	0.2561	0.2432	0.2611
CD (P=0.05)	0.8567	0.5705	0.5419	0.5818
CV	2.46	0.89	0.70	0.97

*Mean of eight replications; Figures in parentheses are arcsine transformed values

In a column, means followed by the same letter(s) are not significantly different by LSD (P=0.05)

Table 2. Emergence of *T. chilonis* on eggs of *C. cephalonica* as influenced by hexane extracts of *H. armigera*

Insect samples	% emergence *
Male whole body	39.33 (38.84)[b]
Female whole body	48.16 (43.95)[a]
Frass extract	25.94 (30.62)[d]
Larval extract	27.83 (31.84)[c]
Control (Hexane)	22.15 (28.08)[e]
Mean	32.68 (34.86)
SEd	0.2559
CD (P=0.05)	0.5702
CV	0.90

*Mean of eight replications; Figures in parentheses are arcsine transformed values

In a column, means followed by the same letter(s) are not significantly different by LSD (P=0.05)

Table 3. Predation by *Chrysoperla zastrowi sillemi* on eggs of *C. cephalonica*, as influenced by hexane extracts of *H. armigera*

Insect samples	% predation after 24 h*
Male whole body	54.37 (47.51)b
Female whole body	61.13 (51.43)a
Frass extract	41.27 (39.97)d
Larval extract	43.93 (41.51)c
Control (Hexane)	37.85 (37.97)e
Mean	47.71 (43.68)
SEd	0.2394
CD (P=0.05)	0.5334
CV	0.67

*Mean of six replications; Figures in parentheses are arcsine transformed values

In a column, means followed by the same letter(s) are not significantly different by LSD (P=0.05)

of female whole body, followed by hexane extract of male whole body (54.37%), larval extract (43.93%) and frass extract (41.27%).

DISCUSSION

Parasitoids detect chemical cues that are emanating from the host insects which help in their host location. These semiochemicals which are often found in the host insect or their by-products act as arrestants or stimulants to the parasitoids to intensify their search in the near vicinity of the host (Tumlinson *et al*., 1992). These findings are in agreement with the report of Lewis *et al*. (1972) who confirmed the presence of host searching stimulant for *T. evanescens* Westwood in scales left by ovipositing corn ear worm moth, *Heliothis zea* (Boddie). Moth scales of *H. zea* and tricosane acted as releaser for the parasitoids, *T. pretiosum* and *T. acheae* and doubled the rates of parasitization by them on *H. zea* eggs over that of unstimulated parasitoids. Saturated long chain hydrocarbons present on the body surface of *H. armigera* and *C. cephalonica* moths were reported to elicit kairomonal response on *Trichogramma* spp. (Padmavathi and Paul, 1997). However, egg wash of *Chilo partellus* (Swinhoe) was reported to increase the parasitoid activity index and per cent parasitism of *T. chilonis* than female and male whole body wash (Paramasivam *et al*., 2004).

In the present study, other than whole body hexane wash, larval and frass extracts of *H. armigera*, could also elicit kairomonal effect towards the parasitoid on the eggs of *C. cephalonica*. But in general, larval and frass extracts of lepidopteran insects were reported to evoke the response of the larval parasitoids as suggested by several workers including, Hu and Chen (1987) and Parthiban *et al*. (2015). The result is in conformity with the findings of Singh *et al*. (2005) who stated that an analysis of *H. armigera* whole body wash for possible kairomonal substances using gas chromatography confirmed the presence of fifteen saturated hydrocarbons, which include, heneicosane and hexacosane. Rest of the saturated hydrocarbons were heptadecane, nonadecane, hexadecane and pentadecane and tricosane which might be reason for enhanced parasitism, emergence and predation.

The significance of these kairomonal substances in behavioural manipulation of entomophagous insects was earlier emphasized and reviewed by Lewis *et al*. (1976). Paul *et al*. (2002) proved beyond that pentacosane and hexacosane recorded very high parasitoid activity index and parasitism for *T. brasiliensis* and *T. exiguum* indicating high kairomonal activity. Srivastava *et al*. (2008) found that kairomones from male *S. litura* and female *S. exigua* showed the highest parasitoid activity index (PAI) and parasitism by *T. chilonis*.

Attraction of *T. chilonis* was more towards female body wash of *Chilo partellus* (Swinhoe), *Sesamia inferens* Walker and *Sitotroga cerealella* Oliver compared to male body wash (Padmavathi and Paul, 1997). The whole insect body of *E. vittella* was found to increase parasitoid activity index and per cent parasitism by *Trichogramma* spp. which may be attributed to the presence of various saturated hydrocarbons in the range of C_{13} to C_{30} with varying quantities (Mahesh *et al*., 2012). Presence of single chain hydrocarbons like dotriacontane and nonadecane would have been responsible for the enhanced predatory activity of *C. carnea*, as suggested by Singh and Paul (2002). Bakthavatsalam and Singh (1999) exemplified scales and abdominal tip extracts of *C. cephalonica* and *H. armigera* elicited good behavioural response in *C. zastrowi sillemi* larvae. Hegde *et al*. (2000) noticed the grub of *C. zastrowi sillemi* to spend the longest time (0.98 min.) near wax droplets smeared with *H. armigera* scale extract, followed by *H. armigera* egg extract (0.54 min.) and abdominal tip extract (0.34 min.). Larvae of the generalist predator *C. zastrowi sillemi* have specific preference to certain hydrocarbons and other chemicals at a particular concentration. Such preferential behaviour of the larvae may be utilized for their activity of manipulation in the release programmes to enhance their host searching activity.

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Mite pests of vegetable crops under protected cultivation in Kerala

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ABSTRACT: Survey conducted to document the diversity of mite pests and their natural enemies associated with vegetables grown under polyhouse, recorded tetranychids - *Tetranychus truncatus* on cucumber and amaranthus, *T. urticae*, *T. okinawanus* and *Eutetranychus orientalis* on cucumber and *T. macfarlanei* on cowpea and French bean and tarsonemid, *Polyphagotarsonemus latus* on cowpea, chilly, tomato, capsicum, cucumber, bittergourd and amaranthus. Insect predators - *Stethorus pauperculus, Oligota* sp., *Scolothrips* sp. and an unidentified species of Cecidomyiidae and predatory mites - *Neoseiulus longispinosus, Amblyseius paraaerialis, Tydeus gossabaensis, Agistemus garrulus, Cunaxa* sp. and *Cheyletus* sp., as natural enemies.

KEYWORDS: Protected cultivation, spider mites, natural enemies

Polyhouse cultivation is being promoted in a big way in the state by providing 75 per cent of the cost as subsidy to the farmers and now there are more than 600 polyhouses in Kerala for vegetable cultivation. The climate inside the polyhouse is very much suitable for the rapid development and multiplication of pests especially the sap feeding species. The most notorious among the sucking pests affecting polyhouse vegetables are the mites. Due to their small size, mites are often overlooked on crops at early stage of infestation. Short life cycle and high fecundity of mites along with the conducive microclimate inside the polyhouse often lead to heavy population buildup of mites on vegetable crops in polyhouses. Farmers usually resort to application of synthetic acaricides for mite management in polyhouses which results in resurgence and residue problems. In this context, the present study was carried out with the objective of documenting the diversity of major species of mite pests and their natural enemies on vegetable crops grown in polyhouses of Kerala.

The work was carried out during 2013-2015 to explore the species diversity of mites and their natural enemies associated with major vegetable crops grown under protected cultivation in Kerala. Random roving surveys were carried out in the polyhouses and rain shelters located in five districts of Kerala, namely Thrissur, Palakkad, Wayanad, Trivandrum and Ernakulam to collect phytophagous mites and their natural enemies on major vegetable crops *viz*., cucumber, cowpea, chilly, tomato, capsicum, cabbage, cauliflower, bitter gourd, French bean and amaranthus. Mite infested leaf samples were collected in polythene bags from randomly selected plants representing different vegetable crops from each polyhouse and brought to the

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laboratory. In the laboratory, the leaves were observed under stereomicroscope and mite specimens were collected using camel hair brush and preserved in 70 per cent ethyl alcohol with a few drops of glycerol taken in glass vials of 1.5ml capacity and labeled. The prey and predatory mites collected in the survey were mounted in Hoyer's media to prepare permanent slides, labeled and numbered serially for identification. The permanent slides prepared were observed under phase contrast microscope for species determination. The insect predators associated with the mites were also collected in polybags and brought to the laboratory where they were examined under stereo binocular microscope and identified.

Six species of phytophagous mites belonging to two different families namely, Tetranychidae and Tarsonemidae were recorded from different vegetable crops grown under protected cultivation

Table 1. Mite pests of vegetable crops in polyhouse

Mite species	Family	Host	District
Tetranychus urticae Koch	Tetranychidae	Cucumber	Thrissur, Wayanad
Tetranychus truncatus Ehara	Tetranychidae	Cucumber, Amaranthus	Thrissur, Palakkad, Wayanad, Ernakulam, Trivandrum
Tetranychus okinawanus Ehara	Tetranychidae	Cucumber	Thrissur
Tetranychus macfarlanei Baker and Pritchard	Tetranychidae	Cowpea, French bean	Thrissur, Wayanad
Eutetranychus orientalis (Klein)	Tetranychidae	Cucumber	Thrissur
Polyphagotarsonemus latus Banks	Tarsonemidae	Chilli, capsicum, tomato, cowpea, cucumber, bitter gourd	Thrissur, Trivandrum, Wayanad, Ernakulam

Table 2. Natural enemies of mite pests of vegetable in polyhouse

Species	**Family**	**Order**	**Host**
Stethorus pauperculus (Weise)	Coccinellidae	Coleoptera	Cucumber, amaranthus
Oligota sp.	Staphylinidae	Coleoptera	Cucumber, amaranthus
Scolothrips sp.	Thripidae	Thysanoptera	Cucumber, amaranthus
Unidentified	Cecidomyiidae	Diptera	Cucumber, amaranthus
Neoseiulus longispinosus (Evans)	Phytoseiidae	Mesostigmata	Cucumber,cowpea, amaranthus,
Amblyseius paraaerialis (Muma)	Phytoseiidae	Mesostigmata	Cucumber, cowpea, amarnathus
Agistemus garrulus (Chaudhari)	Stigmaeidae	Prostigmata	Cucumber, cowpea, chilly
Tydeus gossabaensis Gupta	Tydeidae	Prostigmata	Cucumber, cowpea, chilly
Cunaxa sp.	Cunaxidae	Prostigmata	Cucumber, cowpea
Cheyletus sp.	Cheyletidae	Prostigmata	Cucumber

Fig.1a.White speckling on cucumber

Fig. 2a. Infestation of *Polyphagotarsonemus latus* on cowpea

Fig. 3a. Stunted growth and bronzing of terminal leaves in chillies

Fig.1b. Spider mite infestation on cucumber

Fig. 2b. Bronzing and curling of leaves due to *P. latus*

Fig. 3b. Damage by *P. latus* on chillies

in Kerala (Table 1). Four species of insect predators and six species of mite predators were recorded during the study, associated with mite pests of vegetables under protected cultivation (Table 2).

Phytophagous mites: Of the different species of spider mites collected on vegetables, *T. truncatus* was found to be predominant. It was recorded from all the localities surveyed during the study. Its hosts included cucumber and amaranthus. In cucumber, the mite preferred middle and lower leaves and infestation was pronounced during late vegetative stage. White speckling followed by yellowing and drying of the leaves were the associated symptoms (Fig.1a and 1b).

The tarsonemid mite, *Polyphagotarsonemus latus* Banks was recorded in polyhouses and rainshelter on cowpea, capsicum, chilli, cucumber, tomato and bitter gourd. However, severe infestation was found only on cowpea, capsicum and chilli. The mite infestation on tender terminal leaves lead to bronzing, curling and crinkling of terminal leaves followed by stunted growth and failure in flower production. Severe infestation of *P. latus* on cowpea in a polyhouse at Mathilakam, Thrissur district during 2014 lead to complete failure of the crop (Fig. 2a and 2b). Similarly, chilli crop was completely destroyed by the mite species in a polyhouse at Anthikkad, Thrissur district during September, 2015 (Fig. 3a and 3b).

Natural enemies of mites: Four species of insect predators and six species of mite predators were recorded associated with mite pests of vegetables under protected cultivation during the study (Table 2).

Phytophagous mites are now becoming aggressive pests of most crops especially vegetables. The survey revealed six species of phytophagous mites infesting crops under protected cultivation. *T. truncatus* was first recorded in India from the Northwestern Himalayan regions of Jammu and Kashmir and Himachal Pradesh on *Dahlia* sp. (Rather, 1983). Later, the mite species was reported from Karnataka infesting mulberry leaves (Srinivasa *et al*., 2012). During the study, *T. okinawanus* was

also recorded on cucumber. *T. truncatus* and *T. okinawanus* were reported from Kerala only very recently (Bennur *et al.*, 2015 and Lenin *et al*., 2015). The two spotted spider mite, *T. urticae*, which was reported as the predominant species of mite infesting different vegetable crops of Kerala (Binisha and Bhaskar, 2013) was recorded in the study only on cucumber from Thrissur and Wayanad districts. *T. macfarlanei* was recorded on leguminous crops, cowpea and French bean. It was first reported from India during 1975 on brinjal (Pande and Yadava, 1976). Later, it was reported as a new pest of medicinal plants namely, *Clitoria ternatea* L. and *Justicia adhatoda* L. Nees. from India (Gupta, 2005). The mite species is now emerging as a major pest on a wide range of hosts, many of them being economically important (Ullah and Gotoh, 2013). *P. latus* was reported on a wide range of host plants belonging to more than 60 families. The vegetable hosts reported include chilli, beans, cowpea, cucumber, capsicum, brinjal, potato and tomato. The economic yield loss due to the broad mite was estimated to be 11 to 75 per cent in chilli (Dhandapani and Jayaraj, 1982).

The grubs and adults of *S. pauperculus* and *Oligota* sp. preyed on different stages of tetranychid mites. *S. pauperculus*, *Oligota* sp. were reported to be the efficient predators of spider mites in Coimbatore. Adult of *S. pauperculus* and the grub of *Oligota* sp. consumed maximum number of *T. urticae* in the laboratory (Jeyarani and Ramaraju, 2012). *N. longispinosus* is a potential predator of tetranychid mites, which can be successfully used for its management, especially under protected cultivation. The mass rearing techniques has been standardized by rearing *T. urticae* on bean plants (Sharma and Chauhan, 2013). The number of available natural enemies is considerable for developing an alternative management strategy of mite management for polyhouse crops. Early detection of mites is the most significant decision in the management strategy. Regular monitoring of the crop would help in early detection and there by timely intervention of management methods.

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Redescription of *Achaea janata* (Linnaeus, 1758) with additional sexual dimorphic and structural characters

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ABSTRACT: *Achaea janata* was examined thoroughly for morphological characters. Legs of adults and abdominal segments of larvae and pupae can be used for differentiating male and females of *A. janata.* Spines on distal end of fore femur in male is reported. Characters like endoskeletal structures of thorax in adults, post genitalial segments of larva, genitalial segment of pupa and abdominal appendages of adults are discussed. © 2016 Association for Advancement of Entomology

Keywords: Sexual dimorphism, legs, morphological characters, *Achaea janata*

INTRODUCTION

Acahea janata (Linnaeus, 1758) is a major pest of many agriculturally important crops including castor and tomato. The genus is diverse in the old world tropics, with segregation into African and Indo-Australian to pacific subgroups (Holloway, 2005). Four species of Indo-Australian subgroup have been reported from southern India (Sivasankaran *et. al.*, 2012). Of all, *A. janata* is most commonly occurring pest throughout India due to its wide distribution and host range. The genus *Achaea* Hubner, 1823 belong to tribe Poaphilini of subfamily Erebinae (Zahiri *et al.*, 2012). Understanding of Noctuoidea at higher level (subfamily and tribe) require type characters (Wing pattern or shape, antennal structure and eye size etc.) and other structural characters (Fibiger and Lafontaine, 2005). Sexual dimorphism in *A. janata* is indistinct due to similarities and uniform forewing faciation compared to other genera in the *Achaea/ Parallelia complex* (Edwards, 1978; Holloway, 2005). Although strenuous efforts on managing the pest were carried out widely, works on morphological observation of non type characters are limited. The present study emphasize the detailed morphological characters of *A. janata* collected from different locations of Tamil Nadu and Andhra Pradesh, which include both structural characters of adults and sexual dimorphic characters on all the life stages of this hazardous pest.

MATERIALS AND METHODS

Insect materials observed: 11 ♂ & 7 ♀ 2.ii.2016 Hokenakkal, Tamil Nadu; 13 ♂ & 9 ♀ 18.i.2016 Pollachi, Tamil Nadu; 6 ♂ & 13 ♀ 15.ix.2015 Yercaud, Tamil Nadu; 1♂ & 2 ♀ 22.xii.2015 Periyakulam, Tamil Nadu; 8 ♂ & 3 ♀ 14.xii.2015 Ooty, Tamil Nadu; 18 ♂ & 11 ♀ 30.x.2014 Anaikatti, Tamil Nadu; 24 ♂ & 17 ♀ 21.x.2014 Coimbatore. Tamil Nadu;12♂ & 8♀ Tirupathy, Andhra Pradesh; 9♂ & 11♀ Bapatla, Andhra Pradesh.

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Examination of morphological characters of *Achaea janata* were carried out at Biosystematics laboratory, Department of Agricultural entomology, TNAU, Coimbatore. Adults were collected by use of 125V mercury vapour lamps and lab reared specimens were used for examination of both structural and sexual dimorphic characters. Twenty numbers each of field collected and laboratory reared larvae and pupae were also examined for sexual dimorphic characters. Larvae at 4th and 5th instars were used for examination. Adult moths were subjected to whole body slide mounting with slight modifications in proposed procedure of Sangmi Lee and Richard L. Brown (2006) to fit larger moths for observation of structural characters. Morphological characters were examined with Leica MZ16 Stereomicroscope and Photographs were taken through Leica MZ16 Stereomicroscope equipped with DFC 295 digital camera (LAS 3.8. version 2011). A total of 93 males and 70 females of adults were sexed using the characters of forelegs, Middle legs, wing coupling apparatus and abdominal characters. Species confirmation was done using the keys of Edwards (1978). Terminology proposed by Klots(1970) for genitatial morphology and Kitching and Rawlins(1999) for endoskeletal structures and noctuoid tympanum has been used in the present study for nomenclature purpose.

RESULTS AND DISCUSSION

Adults are uniformly distributed throughout all the regions of India.

Diagnosis: Forewing faciation uniform throughout the genus Wing span 50-56mm**.** Forewing pale brown to dark brown in colour (Fig.1a). Male and female are similar in coloration. Basal, antemedial and post medial lines wavy. An indistict subterminal line. Underneath forewing pattern unique. A broad white patch running across from Sc to anal margin with discontinuation at 1A+2A (Fig.1b). Hind wing rounded, black with one white band running diagonally and three separate patches at apical margin. Underneath hind wing brown in colour with distinct dark brown patch surrounded by white marking at tornus.

Genitalia morphology: Male genitalia with symmetrical valves. Uncus curved, prominent pseudouncus and dictinct socii; tegumen unmodified; juxta X shaped; valva smooth, sclerotised towards margins with single coremata, separate costal and saccular process; Rod shaped slender saccular process from base of sacculus; Costal process trifid, symmetrical (Fig.1d). Aedeagus broad at base, with two triangular cornutii (Fig.1e). Female genitalia with ductus bursae wider than long; Carpus bursae divided into two distinct portions;Distal portion globular; Proximal portion dorsoventrally flattened; Signum present; Ductus seminalis opening in proximal portion of carpus bursae. Genital plate present (Fig.1f).

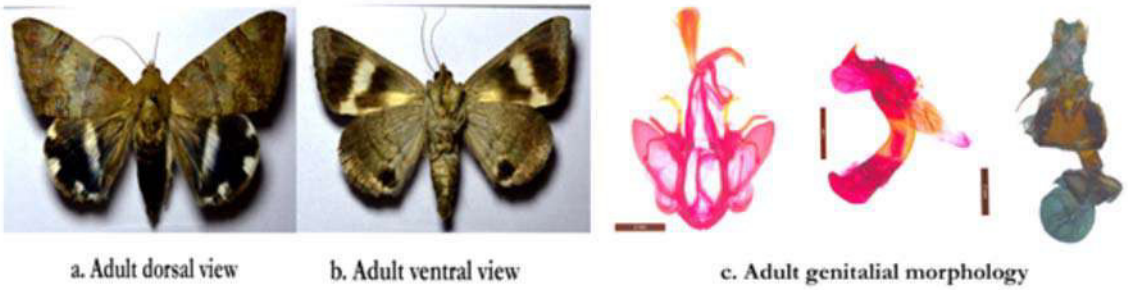
Morphological characters: Filliform antenna; ciliated antinnifers; clypeofrons bare; well proboscis developed with spined tip; Ocelli present; chetostemma absent; scaled upwardly curved labial palpi; foreleg with tibial epiphysis; spined midtibia and unspined hind tibia; Tibial spur formula 0-2-4; (Fig .2).

Structural characters: Prothorax simple; Propleuron unmodified; dorsoventrally flattened rod shaped spina reaching pronotum; (Fig.3a); No distinct cervical sclerites; prothorasic sternal furca separate; Mesothorax elongated; Y-shaped Mesosternal furca (Fig.3b); Mesoscutellum elongated extending beyond metathorasic phragma. Metascutum and metascutellum at similar level; Metathorasic tympanum; Tympanal sclerite broad and flat; Tympanum with four tympanal pockets (I, II, III, IV); Metathorasic phragma overlay tympanal pocket II. Equal sized tympanal Pockets I and II; Pocket IV appearing double (Fig.3c and d); Y shaped metathorasic furca. Alula triangular.

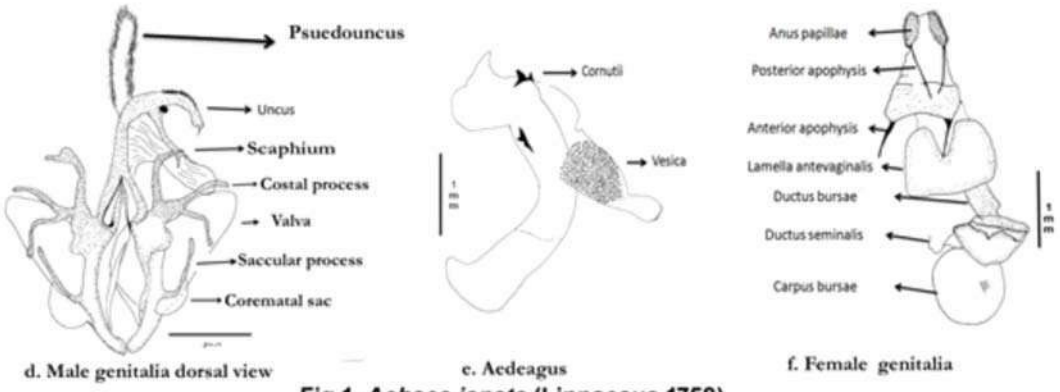
Sexual dimorphic characters:

Wings: Male and female wing pattern similar; Male with single frenulum and bar shaped retinaculum (Fig.4a); Female with two frenulum and a bunch of hair-like retinaculum (Fig.4d).

Legs: Males: Pair of curved spines and hair brushes on outer marginal apex of fore femur (Fig 4b and

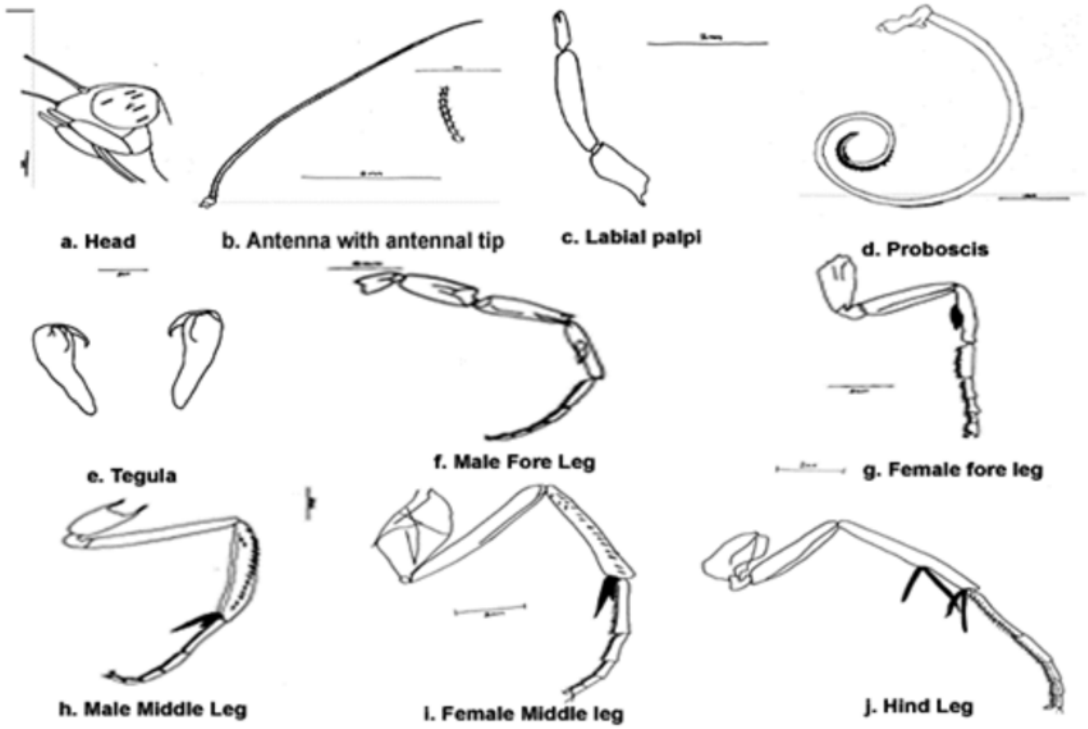


a. Adult dorsal view b. Adult ventral view c. Adult genitalial morphology



d. Male genitalia dorsal view e. Aedeagus f. Female genitalia

Fig.1 *Achaea janata* (Linnaeaus,1758)



a. Head b. Antenna with antennal tip c. Labial palpi d. Proboscis

e. Tegula f. Male Fore Leg g. Female fore leg

h. Male Middle Leg i. Female Middle leg j. Hind Leg

Fig.2 Morphological characters of *Achaea janata*

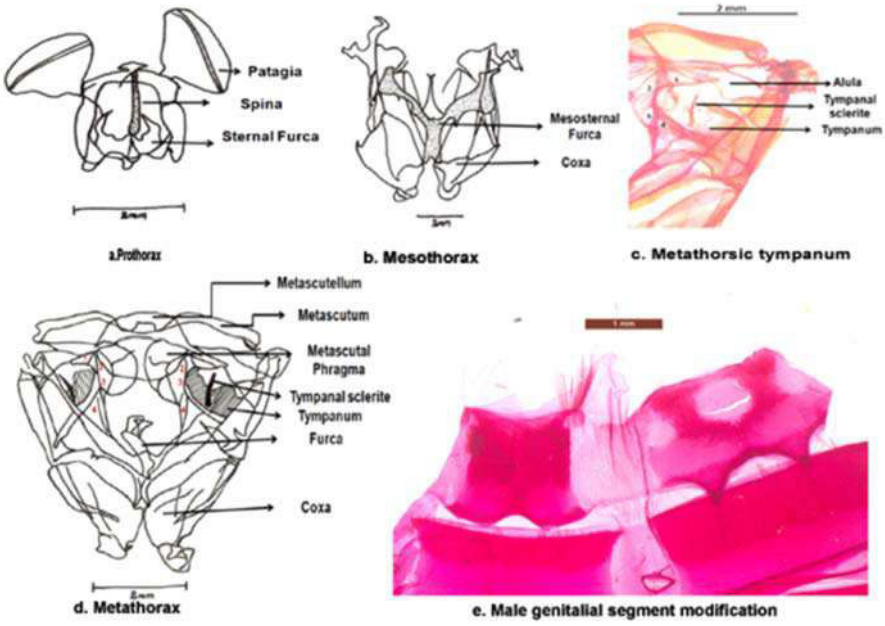


Fig.3 Internal view of thorasic and abdominal segments

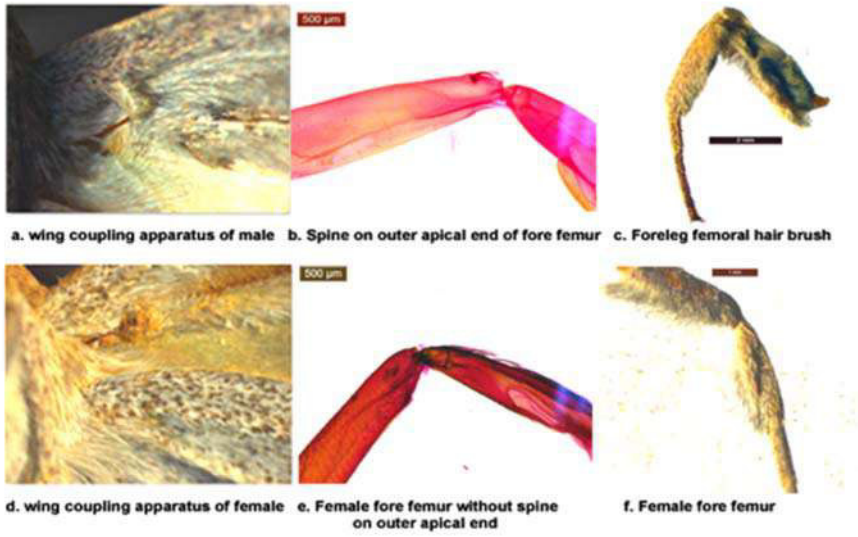


Fig.4 Sexual dimorphic characters in wings and fore legs of adult

c); Outer upper margin of mid tibia grooved with hair pencils (Fig 5a); Females lack hair brushes, spines on fore femur outer marginal and mid tibial groove.

Abdominal segments: Males: sternum of 8th abdominal segment modified with outwardly protruding Posterior lobes (Fig.3e); Hairpencils on 9th abdominal segment of males (Fig. 5c); Females: medially clefted rectangular lodix on 7th abdominal sternum covering the ostium (Fig. 5f).

Immature stages: Pupa**:** anal slit on 10th abdominal segment; Genital opening on 9th abdominal sternum in male; Genital opening on 8th abdominal sternum in females (fig.6b& c); Larva: Females of late

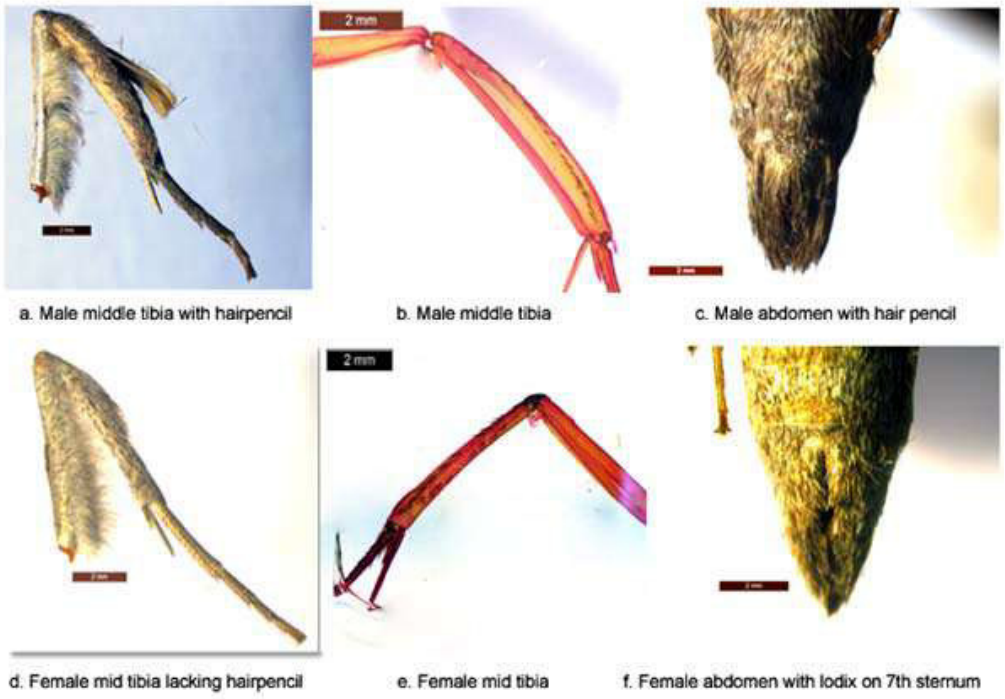


Fig.5 Sexual dimorphic characters in middle leg and abdominal segments of adult

instar(IV and V) larvae with two pairs of translucent pits(Fig.6a) on postgenital segments (i.e.,10 and 11 abdominal ventrum).

Structural characters like noctuoid tympanum with special emphasis on Notodontidae and arctiid/ noctuid families was summarised by Kitching and Rawlins (1999). However tympanal Pocket IV was of phylogenetic importance and is V- shaped in quadrafid noctuoids (Fibiger and Lafontaine, 2005). Small size of tympanal pocket IV in relation to other pockets, double pocket of IV and flat tympanal sclerite observed in *A. janata* confirm its placement in quadrafine noctuoids as these are reported as shared characters of tribe poaphilini and ophiusini of Erebinae (Dombroskie, 2011).

Wing venation, general morphology and genitalial morphology of *A. janata* along with three other species of same genus were described earlier (Edwards, 1978). Taxonomic importance of adult sexual dimorphic characters like mid-tibial groove, modification of eighth abdominal segments and frenulum form were already reported by several authors (Fibiger and Lafontaine, 2005; Edwards, 1978; Holloway, 2005). Spines at distal end of fore femur were observed as additional sex specific character in the present study. This can be compared to forefemur brush of genus *Zale* Hubner belonging to same tribe poaphilini. However forefemur hair brush of genus *Zale* Hubner is similar to mid tibial hair brush of tribe ophiusini. The current character studied (forefemur hairbrush) support the close relationship of tribe ophiusini and poaphilini (Fibiger and Lafontaine, 2005; Zahiri *et al.,* 2012). Fore femur spine distinguishes *A. janata* from other closely related moths of other tribes *viz*., ophiusini, panopodini and catocalini. Fuctional adaptation for presence of fore femur spines and brushes needs further investigation in other tribes and genera.

Larval sexual dimorphism with the help of sex specific translucent pits was studied by several authors throughout the order Lepidoptera (Hinks, C.F and J. R. Byers, 1973; Linda, 1982). Live larvae can be sexed easily at later stages where as earlier stages can be sexed only after staining and preparation (Underwood, 1994). Genital segments

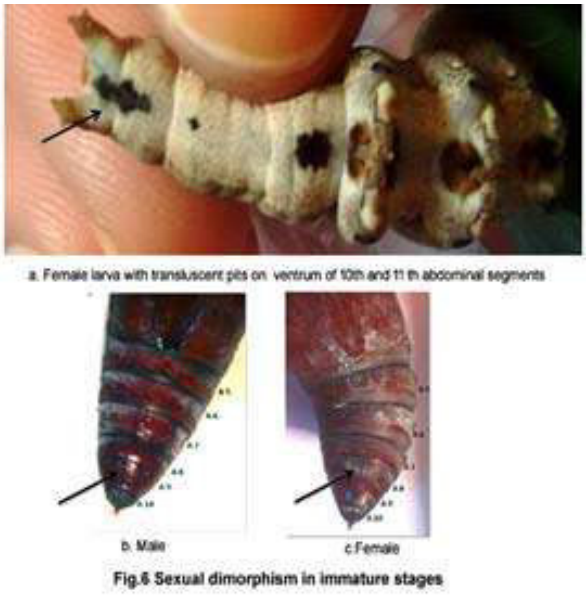


Fig.6 Sexual dimorphism in immature stages

in pupa and adult abdomen were commonly used for sexing. (Muraleedharan and Muraleedharan, 1989).

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Molecular probe, colony structure and SEM of antennal sensillae substantiate intermediate workers of *Oecophylla smaragdina* (Fab.) as typical worker

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ABSTRACT: The polymorphic colony of arboreal weaver ant *Oecophylla smaragdina* Fabricius has three categories of workers designated as typical, major and minor. The colony structure fluctuated sharply with seasons but the numerical ratio of worker castes always remained as 65:25:10. Typical workers have highest number of sensillae/unit area on the terminal segment of antenna and all the above characters established their role in the colony different from major and minor workers. Microsatellite DNA analysis of worker castes indicated high degree of genetic diversity, heterozygocity and genetic polymorphism among three worker castes, reproductive males and females. Mitochondrial DNA analysis proved that all the three categories of worker castes were developed from eggs of a single queen.

KEY WORDS: *Oecophylla smaragdina*, typical worker, molecular probe, SEM antennal sensillae

INTRODUCTION

The weaver ant *Oecophylla smaragdina* Fab. forms large arboreal colonies in Tropical Asia and Australia. It represents a spectacular example for eusocial complexity and plays important role in our ecosystem as an aggressive predator, scavenger and symbiont (Holldobler and Wilson,1983). Adults and brood of these ants form an unconventional, cheap, highly nutritious food and a good medicine among ethnic communities all over the world (DeFoliart,1992) and also among tribes of Kerala (Vidhu and Evans, 2015). Their caste system contain three different types of apterous workers, winged males and winged females. Workers differ in their size, body proportion and in their tasks. The major workers do most of the external work for the colony, such as nest building, foraging, defending and exploring new territory. The minor workers are responsible for taking care of the eggs and young larvae (Holldobler and Wilson, 1990).

Both morphology and internal factors like physiological state and genetic makeup influence division of labour among colony and behavioural specialisations. Genetic studies on social insect groups strongly support the relationship between colony diversity, task specialisation and colony efficiency. Even though many reports have shown that *O. smaragdina* possessed only two types of worker castes (Holldobler,1983; Holldobler and Wilson,1977; Holldobler and Wilson1990; Lokker,1986) we could identify a third category of worker caste with clear difference in morphology and genetic makeup and it was termed as intermediates (Vidhu and Evans, 2011a). Body size, protein profile in SDS-PAGE, amount of formic acid in their poison gland (Vidhu and Evans, 2011b) and

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volatile compounds in their Dufour,s gland (Vidhu and Evans, 2015) have very well attested that the intermediates are unique and distinct from other two worker castes. Molecular probes, study of colony structure and SEM of antennal sensillae on the intermediate workers were under taken and the results are presented in this paper.

MATERIALS AND METHODS

Nests from a single colony were collected from the University College Campus, Thiruvananthapuram and anaesthetised. Three types of workers were separated, washed in distilled water, blotted with tissue paper and used for the study.

Colony structure: Large permanent nests of *O.smaragdina* of approximately 20 cm diameter were plucked from the tree with the branch itself and immersed in a wide mouthed jar containing cotton soaked in chloroform. After10 minutes, the dead ants were transferred in to a tray. Each type of ants was separated and counts were recorded.

Antennal sensillae studies of different worker castes: Morphology of antennae of all different individual types of the colony was observed using binocular dissection microscope with magnification of 4x. (Magnus, MS 24,India) and photographic images were recorded. Scanning Electron microscopic pictures of terminal segment of antennae of the three types of workers were taken. Sensillae identification was made as described by Gullan and Craston, 2005; Martin *et al.,*2011; Hartenstein, 2005 and Euzebio *et al.,* 2013. Distribution of sensillae on terminal segment of antennae was studied in all the castes by counting number of each type of sensillae per 60 µm × 60 µm area.

Scanning Electron Microscopic analysis (SEM): Antennae were cut and fixed in 3% glutaraldehyde buffered with 0.1 M phosphate buffer at room temperature or 0-4^0 C (minimum 2-4hours or maximum 24-48hours). The fixed sample was immersed in 1-2% Osmium tetroxide in 0.1 M phosphate buffer pH 7.2 (2-4 h) at room temperature and in an opaque container. Washed in 0.1 M phosphate buffer pH 7.2 (3 x 10 min.). Dehydrated in grades of ethanol (15-30 min each).Critical Point Drying was done. The antennae were then mounted on a brass stub and Gold sputtered for 2 min (SPI-Module Gold Sputter Coater).Observations were made using a JEOL JSM-5800VL SEM.

Microsatellite DNA Fingerprinting: Microsatellite DNA finger printing for 5 microsatellite loci (Table.1) was done (Shluns *et al*., 2011; Shimizu *et al*., 2002; Schuelke, 2000) in three types of workers collected from 10 permanent nests in a single colony of *O. smaragdina*. Genomic DNA was isolated from single specimens using DNeasy® Blood and Tissue Kit (Qiagen) following manufacturer's instructions. Agarose Gel Electrophoresis for DNA Quality check was done.PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X PCR buffer (contains 1.5 mM $MgCL_2$), 0.2mM each dNTPs (dATP, dGTP, dCTP and dTTP), 10ng DNA, 0.4 µl of PhireHotStart II DNA polymerase enzyme (Thermo scientific), 0.1 mg/ml BSA, 1pM of M13-tailed forward primer, 5 pM of reverse primer and 5pM of FAM-modified universal M13 primer. The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). For capillary Electrophoresis of PCR Products ,one micro litre of the PCR product was added to 10 ìl Hi-Di formamide (Applied Biosystems) and 0.5 ìl Gene Scan 500 Liz - Size Standard (Applied Biosystems) and run on the ABI 3500 Genetic Analyzer. Data was analysed using Gene Mapper ID-X v1.4 software.

Allele frequency including allele number, inbreeding coefficient heterozygosity, gene diversity, polymorphism information content (PIC), frequency based genetic distance and stepwise patterns for microsatellite data were calculated using Power Marker® software.

DNA barcoding using universal primers of Cytochrome oxidase 1 (Cox1): Genomic DNA was isolated from single specimens using DNeasy® Blood and Tissue Kit (Qiagen) following manufacturer's instructions. Thorax of 3 types of workers were taken as sample by removing the head and abdomen using a sharp blade .The tissue were cut into small pieces and placed in a 1.5 ml

micro centrifuge tube. 180 µl of ATL buffer and 20 µl of proteinase K was added and incubated at 56 °C in a water bath until the tissue were completely lysed. After lysis, 5 µl of RNase A (100 mg/ml) was added and incubated at room temperature for 5 minutes. 200 µl of AL buffer and 200 µl of 100% ethanol was added and mixed thoroughly by vortexing. The mixture was pipetted into DNeasy Mini spin column placed in a 2 ml collection tube and centrifuged at 8000 rpm for 1 minute. The DNeasy mini spin column was transferred to a new 2 ml tube and washed with 500 µl of AW1 buffer. Washing step was repeated using AW2 buffer. After washing the DNeasy mini spin column was placed in a clean 1.5 ml tube and DNA was eluted out using 50 µl of AE buffer. Agarose Gel Electrophoresis for DNA Quality check was done. The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X PCR buffer (150mM TrisHCl , pH-8; 500mM KCl), 0.2mM each dNTPs (dATP, dGTP, dCTP and dTTP), 2.0mM $MgCl_2$, 20ng DNA, 1 unit of AmpliTaq Gold DNA polymerase enzyme, 0.15 mg/ml BSA and 3% DMSO, 0.5M Betaine, 5 pM of forward and reverse primers (Folmer *et al.,* 1994).

After Agarose Gel electrophoresis of PCR products, ExoSAP-IT Treatment was done. Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems , USA) following manufactures protocol. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1.

Statistical analysis: Analysis results were done using Microsoft excel tools. Microsatellite data analysis was done using Power Marker soft ware.

RESULTS

Major workers are the largest among the workers with body length ranged from 10.0 to 11.0 mm with mean body weight of 11.8 mg and it will be clear from Table.II and Figure.1a. They constituted 25% of total number of worker ants in a permanent nest.

Table.1.Primers used for Microsatellite Analysis

No.	Name	Sequence
1	MS6.7F	**TGTAAAACGACGGCCAGT**AGAGGGCACACATCCAACTAC
	MS6.7R	CATCGTAAGGAGAATTTTCGT
2	MS7.4F	**TGTAAAACGACGGCCAGT**ATTGCCGAGTGAAAGAGGAAC
	MS7.4R	AACTTCGCAGAATGACGAGTC
3	Osma101F	**TGTAAAACGACGGCCAGT**ACCTTACGATCGTGGCAG
	Osma101R	AATACTCCGTGACAATCC
4	Osma37F	**TGTAAAACGACGGCCAGT**GAATCCAGACCCGACGAACG
	Osma37R	CGAGAATCCGCCGCAATGAC
5	Ccon70F	**TGTAAAACGACGGCCAGT**GCATTAAAGTCGGGACGGAC
	Ccon70R	CAGATGCGAAGAGCTCGC

*Note: Forward primers are M13-tailed (**in bold**)*

Table 2. Primers used

Target	Primer name	Direction	Sequence (5' → 3')
COX1	LCO	Forward	GGTCAACAAATCATAAAGATATTGG
	HCO	Reverse	TAAACTTCAGGGTGACCAAAAAATCA

The ratios of the length of head with thorax or head with abdomen were around the value of 0.5. (Table.3).Almost 65 % of worker ants in a colony was found to be intermediate forms (typical worker) and were found actively engaged in colony maintenance along with major workers. The typical workers are significantly shorter than the major workers. Their body length ranged from 7.9 to 8.7 mm with mean body weight of 6.3 mg. The ratio of length of head with thorax was below the value of 0.5, but the ratio of the length of head with abdomen was above the value of 0.8 (Table.3). Typical workers were almost double the length of minors and four times the weight of minors. (Table.3, Fig.1b.). Minor workers were very small when compared to major workers. They were mostly confined within the nests and rarely observed in the field. They constitute less than 10% in total number of workers. The body length of minor worker was found to be 4.3 to 4.9 mm (Table.3, Fig.1c).

The colony individuals showed marked difference in the shape, size and number of segments in their antennae (Fig.2). Antennae of three worker castes consisted of total 12 segments such as scape, pedicel and 10 flagellomeres. In major workers antennal length was 7.0 ± 0.2 mm and scape was very long compared to other workers. Intermediate workers possessed a medium sized antennae and length was 5.1 ± 0.1 mm. Minor workers possessed short stout antennae and the shape of terminal segment differed from other worker castes and it was rounded. Its length ranged between 1.9 mm to 2.1mm (Fig.2).

Three different worker castes showed significant difference in the number and distribution of sensillae on the terminal segment of the antennae .Scanning electron microscopic studies on different types of sensillae on the terminal segment of antennae of different castes of *O.smaragdina* has resulted in the identification of four types of sensillae (Fig.3) and they are,

1. Sensilla trichoidea type 1- ST_1
2. Sensilla trichoidea type 2- ST_2
3. Sensilla basiconica- SB
4. Sensilla ampullacea - SA

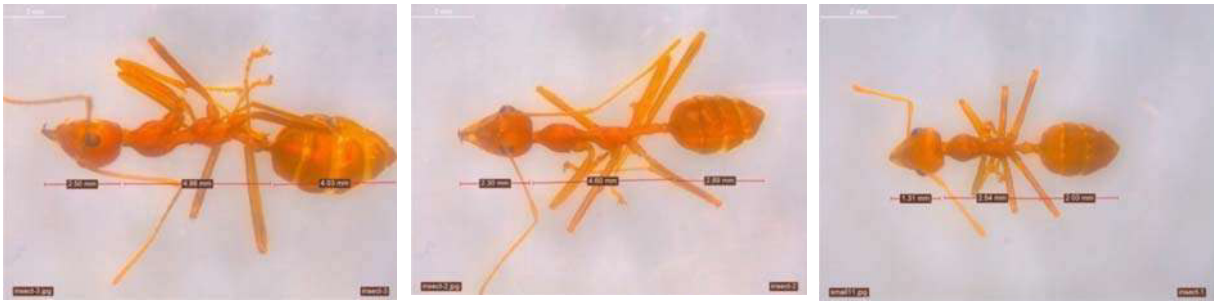
The shafts of Sensilla trichoidea type 1 (ST_1) are long, and narrow and tapering terminally. They vary in thickness, with diameter (near the base) of about 2-3µm and length of 11-13 µm. The Sensilla trichodea type 2 (ST_2) were long, tapered like curved hairs possessing an encircling and a middle

Table 3. Body dimensions of colony individuals of *O.smaragdina*

Sample	Worker castes		
	Major	**Intermediate**	**Minor**
Body length *			
Whole body	10.5 ± 0.5	8.3 ± 0.4	4.6 ± 0.3
Head	2.2 ± 0.2	2.2 ± 0.1	1.2 ± 0.06
Thorax	4.4 ± 0.3	4.2 ± 0.2	2.2 ± 0.1
Abdomen	4.1 ± 0.3	2.6 ± 0.2	1.9 ± 0.1
Body Width *			
Head	1.9 ±0.04	1.6 ± 0.03	1 ± 0.02
Thorax	1.1 ± 0.02	1.1 ± 0.01	0.7 ± 0.01
Abdomen	2.1 ± 0.05	1.7 ± 0.03	1.5 ± 0.01
Body Weight **			
Whole body	--11.8 ± 0.8	6.3 ±0.4	1.3 ± 0.08

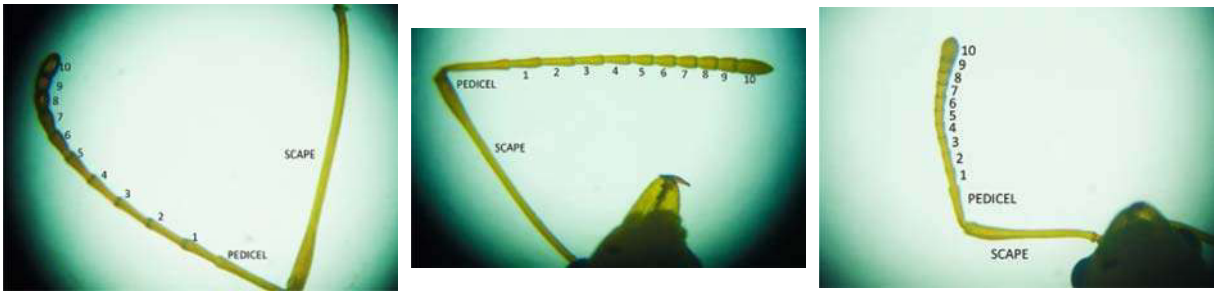
* Value are expressed in millimetre n=10, ± SD

**Value are expressed in milligram, n=10, ± SD



a) Major worker (1x) b. Intermediate worker (1x) c) Minor worker (1.5x)

Figure 1. Three types of worker castes of *O.smaragdina*



a. Major b. Intermediate c. Minor

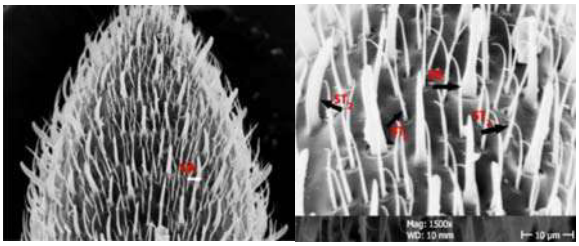
Figure 2. Antennae of three worker castes

cuticular ledges. They vary in thickness, with diameter (near the base) of about 1 to 2 µm and length of 13-14 µm. Sensilla basiconica (SB) type always consisted of two parts, a peg and a socket. The peg was porous on the distal end. They vary in thickness, with diameters (near the base) of about 3-4 µm and length of 13-15 µm. Sensilla ampullacea were characterized by prominent elliptical depression and a central opening and approximately 2 µm diameter.

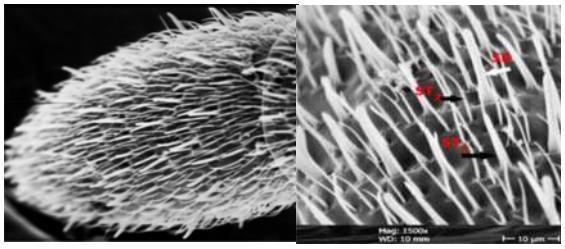
Among the three categories of workers, the most abundant sensilla on the tip of antenna was ST_2 followed by ST_1 and SB and number of sensilla type SA was the least one. The number and distribution of all the above four types of sensilla in three categories of workers, present in unit area, at the terminal antennal segment is shown in Table 4.The three categories of workers showed a significant difference in the number of sensillae among one another with highest density in typical workers. Shape of the terminal segment of antennae of major and typical workers were almost same but the density of sensilla distribution was very high in typical workers than that of major worker (Table.4). The shape of terminal segment of the antennae of minor worker was almost round (Fig.3) but that of other two worker categories, it was pointed.

Microsatellite DNA Fingerprinting: Microsatellite DNA finger printing was done in different colony individuals such as three types of workers, winged males and females of single nests of *O.smaragdina*. Five short sequence repeat markers were used for this study. Based on the microsatellite sequence analysis results, intra nest relatedness and frequency based genetic distance between five colony individuals in the colony were analysed using Power marker soft ware and the results were shown in Table.5 and Table.6. The results have clearly indicated significant genetic diversity and genetic polymorphism among three categories of workers and also between winged females and males.

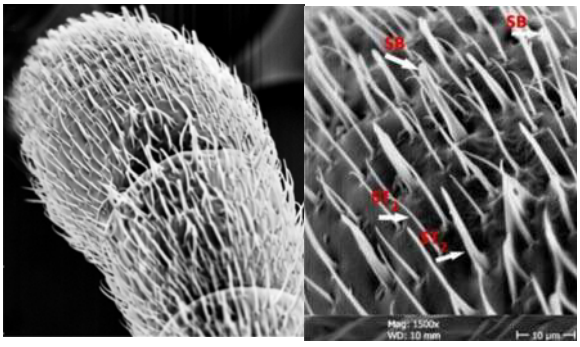
DNA Barcoding using universal primers of Cytochrome oxidase 1 (COX1): Amplified PCR products of universal primers of mitochondrial Cytochrome C oxidase 1 gene from three worker castes showed no significant difference. The Cytochrome oxidase gene sequences of three



a. Major



b. Intermediate



c. Minor

Figure 3. Sensillae on the terminal antennal segment of major, intermediate, minor workers of *Oecophylla*

workers were shown as Fig.4 which showed only single nucleotide substitution in minor workers.

DISCUSSION

Even though previous investigators have described only two categories of workers such as major and minor workers (Holldobler,1983; Holldobler and Wilson,1977; Holldobler and Wilson1990; Lokker,1986),careful observation has revealed that there are three categories of workers such as major, intermediate and minor, and they are exhibiting significant difference in morphology, biochemistry and genetic constitution and are not at all exhibiting, overlapping of body dimension such as length of the body and antennal length. During all seasons the distribution of workers in the colony remained constant with a numerical ratio of 25:65:10 as major, intermediates and minor respectively. Even though the colony structure such as presence of brood and reproductive forms are closely related with rainy season the numerical ratio of the worker castes remained constant throughout the year (Vidhu,2015).The previous investigators have described the intermediate category of workers together with major workers(Holldobler,1983; Holldobler and Wilson,1977; Holldobler and Wilson1990).

All major workers were with body length ranging from 10 to 11 millimetres, intermediate categories were between the length of 7.9 to 8.7 millimetre and minor workers were too much smaller than the other two categories. The three categories of workers showed clear difference in size and body proportions. Poison gland secretion of the three categories of workers showed sharp difference in

Table 4.

Density and distribution of Sensillae on the terminal antennal segments of different colony individuals of *O.smaragdina*

Different castes in the colony	Sensilla trichoidea		Sensilla basiconica SB	Sensilla ampulaceae SA
	ST_1	ST_2		
Major	11 ± 0.5	54 ± 3.2	5 ± 0.03	1 ± 0.01
Intermediate	17 ± 1.5	65± 4.3	8 ± 0.02	1 ± 0.09
Minor	11 ± 0.8	50 ± 3.0	4 ± 0.04	1 ± 0.01

All the values are mean ± SD, n=6.
Number of sensillae at unit area of 60µm×60µmwas presented

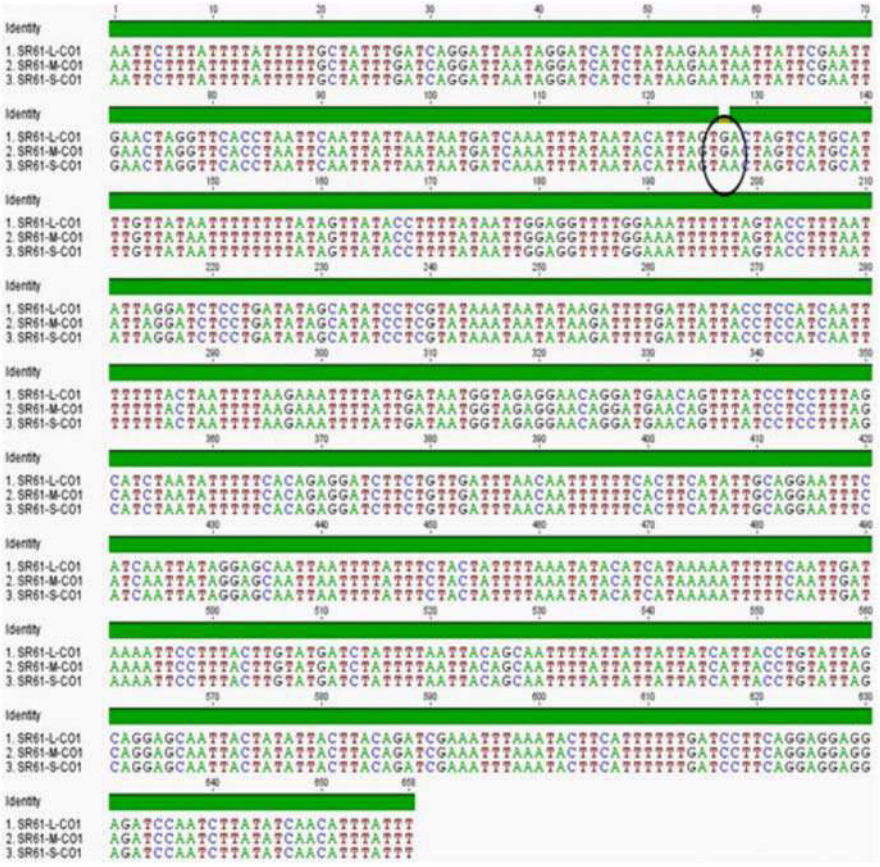


Figure 4. Sequence alignment- Cox 1

the formic acid and the amount of FA vary sharply under different ethological states (Vidhu and Evans, 2014). Analysis of the Dufour'sgland secretion of the three categories of workers proved the contrasting difference in the presence of 23 chemical compounds inintermediates,13 in major and 17 in minor workers (Vidhu and Evans,2015). Gel doc analysis of the protein profile of the head and thorax of three categories clearly showed marked difference in molecular weight of different bands. The head of major worker showed 13 bands, intermediates with 23 bands and minor with least number of bands as 11 (Vidhu, 2015). Presence of highest number of volatile compounds in intermediate category of workers showed their difference in role in the colony in communicating within colony mates and presence of additional enzyme system in then for the production of additional volatile compounds. The total protein content of three categories of workers showed significant variation and its content was highest in intermediates and lowest in minor workers. Electropheretic profile also showed sharp difference in head and thorax of three categories of workers. The intermediates even though appeared as miniatures of major workers they differed sharply in the quantity and quality of body proteins. Differential distribution of protein in the head and thorax of intermediates and major workers clearly indicated their genetic dissimilarity. The total content of protein was highest among intermediates and lowest among minor workers. This also supported the argument that the worker types in *O.smaragdina* colony are not two but three distinct categories (Vidhu and Evans,2011a). Lokkers (1986) has reported that the first few batches of eggs laid by the newly impregnated queen were developed in to small ants which are smaller than major workers and larger than minor workers. This was very well attested by the investigators that the dealated, green coloured, pregnant queen lived well camouflaged at the tip of the shoot, among tender

Table.5. Microsatellite DNA Fingerprinting- Power Marker Data

Marker	Major allele frequency	Allele no.	Gene Diversity	Heterozygosity	PIC	Inbreeding coefficient (f)
M1	0.4000	4.0000	0.7000	0.8000	0.6454	-0.0323
M2	0.7000	2.0000	0.4200	0.6000	0.3318	-0.3333
M3	0.4000	4.0000	0.7000	0.8000	0.6454	-0.0323
M4	1.0000	1.0000	0.0000	0.0000	0.0000	NaN
M5	0.7000	3.0000	0.4600	0.4000	0.4102	0.2381
Mean	0.6400	2.8000	0.4560	0.5200	0.4066	-0.0297

PIC- polymorphic information content

Table 6. Frequency based genetic distance among colony individuals

OTU	S1	S2	S3	S4	S5
S1	0.0000	0.4495	0.1273	0.5550	0.3521
S2	0.4495	0.0000	0.5322	0.2775	0.3521
S3	0.1273	0.5322	0.0000	0.6376	0.4347
S4	0.5550	0.2775	0.6376	0.0000	0.3750
S5	0.3521	0.3521	0.4347	0.3750	0.0000

leaves and the ants developed from first few batches were identical to intermediate category of workers in size and body proportion.

The antennae form the major sense organs for insect communication and survival, and the antennal sensillae receives stimuli for various behavioural modifications in the host such as mate selection, locomotion, foraging and defence which are in constant contact with the environment (Chapman, 1982). The antennae of all worker castes possessed scape, pedicel and 10 flagellomeres (total 12 segments) .Scapes with flagellomeres constitute antennomeres and thus females possessed 11 antennomeres. The type, abundance and distribution of sensillae on antennae depend on various behavioural aspects (Chapman, 1982). The terminal segment of antennae of different castes of *O.smaragdina* possessed two types of ST (ST_1, ST_2), SB, and SA. Sensillae density on the terminal segment of antennae was maximum in intermediate category of workers and the least in minor workers. The present study showed that minor workers were almost fully confined within the nest itself and were not participating actively with other two categories of workers for maintenance of territory, predation and defending invaders. Differential distribution of sensillae on the antennae of worker castes clearly indicated their dissimilar role in the colony. Different patterns of trichoid and basiconic sensilla numbers were described in different populations of *Rhodnius prolixus* sampled from east and west of the Andes Mountains. These differences suggest that the geographical isolation of the populations was associated with the numbers of antennal sensillae (Esteban *et al*., 2005).Variations in sensory organs between two populations of *Atta robusta* may indicate an adaptation of this species to different environmental conditions (Euzebio*et al.,* 2013).

The different types of sensillae we have identified in *O.smaragdina* fully agreed with the previous studies (Martin *et al*.,2011). Sensilla trichoidea (ST_1,ST_2) was the most abundant sensillae in all the individuals within the colony of *O.smaragdina.* The adult major workers possessed ST_2 density of 60-66 numbers/3600 $\mu m^{2,}$ area at the terminal segment of antenna. Among three types of workers the density of ST_2 on the antennal tip was highest

among in intermediate category of workers among the trichoid sensillae, ST_1 (thick, curved at base) is considered as mechanoreceptors and ST_2 forms gustatory receptors (Baaren*et al*., 2007). The ampullacea sensilla of ant species *Atta* are considered to be associated with the detection of the CO2 concentration within nests (Kleineidam*et al.,* 2000) and these type of sensillae were found to be very less in *O.smaragdina* workers.

The relationship between colony task, body size and lineage appeared to be complex. Colony genetic diversity might improve division of labour by increasing the morphological or behavioural variation among workers (Crozier and Page 1985; Robinson 1992). Studies have been reported on a genetic component to worker size polymorphism observed in ant colonies such as *Formica, Acromeryrmex and Camponotus* (Frazer *et al.,* 2000; Hughes *et al*., 2003). Here the genotyping of three worker categories, for 5 microsatellite loci were done. Good levels of genetic diversity among 3 groups were obtained for 4 loci. The population diversity and allelic variability is indicated by polymorphic information content (PIC). The PICs ranged between 0.000 and 0.6454 with a mean of 0.4066 (Table.5). The data on allele frequency based genetic distance among 5 groups revealed that the typical or intermediate worker group has shown diversity from other groups such as major, minor, winged males and winged females as 0.4495, 0.5322, 0.2775, 0.3521 respectively. Interestingly the major, intermediate and minor groups display significant genetic distance from each other (Table.5, 6). High levels of gene diversity and heterozygosity also indicate genetic variability among each caste.

Cox 1 gene of mitochondrial DNA in *O.smaragdina* workers was more or less similar in mass and sequence data and it has revealed that the three categories of workers not exhibited any significant difference. These data will ultimately aid in investigations on dynamics of morphological and developmental evolution as well as biology of this social insect. Even though all the first few batches of eggs laid by the newly impregnated young queens developed in to small workers of intermediate size (Lokers ,1986),as the queens gradually matured, genetic polymorphism within the genes might have resulted to phenotypic polymorphism among workers.

On the basis of presence of secondary metabolite, formic acid secreted by the poison gland and also on the basis of the presence of volatile compounds in the Dufour's gland, the intermediate category of workers stood between the other two categories of workers such as major and minor as a unique one with special characters. Even though the three categories of workers are developed from the eggs laid by a single mother the intermediate category of workers maintained their own individuality among other workers by possessing certain peculiar features such as highest amount of body protein, significant variation in the amount of formic acid in their poison gland and highest number of volatile secondary metabolites in their Dufour's gland and highest number of sensillae (Vidhu, 2015; Vidhu and Evans, 2015). In our investigation it was well understood that the major population of workers are intermediates and it was always around 65% of the total number. The genetic variability within the different categories of workers has very well attested the existence of an intermediate category of workers which are not mere miniatures of major workers, but as a third category with different mode of chemical communication by Dufour's gland secretion. So it can be interpreted that the division of labour such as looking after the brood, colony maintenance and defence etc. develop only during the establishment of the new colony in to wider territory and original workers are intermediate category and major and minors are secondary modification through genetic polymorphism and differential expression of genes. So the intermediate category of workers can be designated as typical workers.

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Aquatic insects of a tropical rain forest stream in Western Ghats, India

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ABSTRACT: In the studies on diversity, abundance and distribution of aquatic insects in Kallar stream and its tributaries in Western Ghats, collected on a monthly basis from five different sites revealed a total of 13,510 individuals belonging to 9 orders, 61 families and 125 genera. Trichoptera was the most dominant order with maximum number of individuals. It was followed by Ephemeroptera, Odonata, Hemiptera, Plecoptera, Coleoptera, Diptera, Megaloptera and Lepidoptera. Shannon-Weiner, Simpson dominance and Margalef's richness indices were found to be highest in site 5 and lowest in site 3. The most pollution sensitive aquatic insects are high in the main Kallar stream (site 5) compared to the tributaries. In the tributaries many anthropogenic activities are taking place and these factors have direct and indirect impact on the diversity of aquatic insects. So this may be the reason for the low abundance of the pollution sensitive taxa in the tributaries compared to the main Kallar stream.

KEYWORDS: Aquatic insects, Western Ghats, biodiversity indices

INTRODUCTION

Insects are the integral part of any ecosystem and their variety, number, size, life history, food habits, power of adaptation, high rate of reproduction and various modes of locomotion are some of the reasons for the success of this group in influencing the structure and function of terrestrial and aquatic ecosystem (Sundari and Santhi, 2008). Aquatic insects are a group of arthropods that live or spend part of their life cycle in water bodies (Pennak, 1978). More than one million insect species have been described so far, that is over 50% of all known organisms (Segers and Martens, 2005). About 4500 species of insects of the world are known to inhabit diverse fresh water ecosystems (Balaram, 2005). They involved in nutrient cycling and form an important component of natural food web in aquatic ecosystem. These insects are used to monitor the biological integrity of stream ecosystem in various studies (Rosenberg and Resh, 1993). Most importantly aquatic insects are good indicators of water quality since they have various environmental disturbances tolerant levels (Arimoro and Ikomi, 2007). Several orders of insects, especially Ephemeroptera, Plecoptera and Trichoptera (EPT) require high quality water for their existence. Aquatic insects show different modes of existence or habits which include skaters (adapted for life on water surface), swimmers (adapted for fish like swimming), clingers (adapted for attachment to substrate surfaces), sprawlers (inhabiting the surface of floating leaves of vascular plants or fine sediments in depositional habitats), climbers (living

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and moving upward on vascular plants or detrital debris) and burrowers (inhabiting fine sediment) (Morse *et al.,* 1994). In relation to functional feeding groups, invertebrates can be classified as: collectors (gatherers or filterers), shredders, scrapers, and predators (Cummins and Klug, 1979; Merritt and Cummins, 1996).

In spite of some studies carried out on the aquatic insects in various streams of Western Ghats (Sivaramakrishnan and Job, 1981; Sivaramakrishnan *et al.,* 1996, 2000; Anbalagan *et al.,* 2004; Subramanian and Sivaramakrishnan, 2005; Subramanian *et al.,* 2005; Anbalagan and Dinakaran, 2006; Dinakaran and Anbalagan, 2007 a, b, 2008; Dinakaran *et al.,* 2009; Selvakumar *et al.,* 2012), there has not been any attempt to document their diversity in the Kallar stream and its tributaries before. Kallar stream is a typical rain forest stream located in the Southern tip of Western Ghats. 'Kallar' literally means stony river. The present study was carried out to determine the diversity, abundance and distribution of aquatic insects in the Kallar stream and its tributaries.

MATERIALS AND METHODS

Study area

The study stream Kallar is a perennial river located near Ponmudi in Thiruvananthapuram district, Kerala, which forms the upper course of Vamanapuram River, part of Neyyar Wildlife Sanctuary. It originates from Chemmunji Mottai, a mountain peak in the Western Ghats at an elevation of 1860 m above MSL. In this study five collection sites were selected, they are Darpha-Kalungu (S1- 8°40'42se N, 77°04'02se E), Pottanchira (S2-8°41'31se N, 77°03'09se E), Kaliyikkal (S3-8°40'16se N, 77°06'04se E), Meenmutti (S4-8°42'36se N, 77°07'41se E) and main Kallar (S5-8°43'42se N, 77°07'37se E). From these the first four sites are the tributaries of Kallar stream and the fifth one is the main stream. The sites are chosen based on their location relative to habitat availability, land use pattern and human intervention. At each sampling locality, a stretch of 100 m area was chosen for collection of samples.

Field and laboratory methods

Samplings were done on monthly basis from January 2013 to December 2013. Aquatic insects were collected by using kick net (1m² area, mesh size 200 μm) and D-frame net (mesh size 50 μm). The samples were placed in white trays for sorting and screening. The sorted invertebrates were collected without any damage using fine forceps and they were preserved in 70 % alcohol. In the laboratory, the immature insects were sorted, identified and counted under a stereoscopic microscope (Labomed CX Rlll). The collected samples were identified at genus level using published keys (McCafferty and Provonsha, 1981; Morse *et al.,* 1984; Yule and Sen, 2004; Subramanian and Sivaramakrishnan, 2007). All the taxa encountered during the study were assigned a habit (mode of existence) and functional feeding categories with the help of published references (Cummins and Klug, 1979; Merrit and Cummins, 1984; Resh and Rosenberg, 1984; Pringle *et al.,* 1988).

Statistical analysis

One-way ANOVA was performed to study the changes in the insect abundance and diversity across sites (SPSS, 2006). The biodiversity indices like Margalef's richness index, Shannon-Weiner diversity index and Simpson dominance index values were calculated using the software PAST (2005).

RESULTS

A total of 13,510 individuals belonging to 9 orders, 61 families and 125 genera were collected and identified (Table 1). Trichoptera were the most abundant order with the highest number of individual. In Trichoptera the abundant family was Hydropsychidae with seven different genera and the most abundant genus was *Hydropsyche sp.* and the least abundant genus was *Diplectrona sp.* The least abundant families are Psychomyidae and Xiphocentropodidae. In the order Ephemeroptera numerically the most abundant family was Leptophlebidae with four different genera. Among these the most abundant genus was *Thraulodes*

Table I. Abundance of the aquatic insects in the Kallar stream and its tributaries during January 2013 to December 2013

Order	Family	Genus	Site 1	Site 2	Site 3	Site 4	Site 5	Grand Total
EPHEMER	**Leptophlebiidae**	*Leptophlebia sp.*	112	144	26	112	42	436
OPTERA		*Thraulodes sp.*	166	170	109	76	134	655
		Choroterpes sp.	8	1	12	1	9	31
		Hebrophlebiodes sp.	118	110	27	97	31	383
	Ephemeridae	*Ephemera sp.*	12	13	7	8	15	55
	Potamanthidae	*Potamanthus*	1	0	0	0	4	5
		Rhoenanthus sp.	1	0	0	0	0	1
	Ephemerellidae	*Ephemerella sp.*	1	2	0	2	4	9
	Tricorythidae	*Neurocaenis sp.*	0	0	0	0	1	1
	Caenidae	*Caenis sp.*	117	85	51	15	17	285
	Heptageniidae	*Heptagenia sp.*	4	4	1	117	165	291
		Epeorus sp.	0	1	2	51	184	238
		Thalerosphyrus sp.	2	2	0	130	224	358
	Baetidae	*Baetis sp.*	111	72	59	73	49	364
		Cloeon sp.	16	27	9	7	13	72
Total			669	631	303	689	892	3184
Mean ±SE			44.6± 3.35[b]	42.07± 5.64[b]	20.2± 5.00[a]	45.93± 4.10[b]	59.47± 4.57[b]	212.27± 6.58
PLECOPTERA	**Perlidae**	*Neoperla sp.*	91	117	23	239	393	863
		Tetropina sp.	1	0	0	2	0	3
		Perlesta sp.	1	2	5	21	80	109
Total			93	119	28	262	473	975
Mean ±SE			31± 1.93[a]	39.67± 1.81[a]	9.33± 0.6[a]	87.33± 3.12[b]	157.67± 5.01[c]	325± 6.93
TRICHOPTERA	**Hydropsychidae**	*Arctopsyche sp.*	64	85	64	114	114	441
		Parapsyche sp.	20	16	28	26	74	164
		Diplectrona sp.	2	1	2	0	11	16
		Ceratopsyche sp.	1	0	2	14	2	19
		Cheumatopsyche sp.	30	73	34	62	105	304
		Hydropsyche sp.	219	422	263	428	550	1882
		Potamyia sp.	1	1	4	4	9	19
	Polycentropodidae	*Polycentropus sp.*	1	7	2	39	58	107
		Nyctiophylax sp.	0	1	0	1	5	7
	Psychomyeidae	*Psychomyia sp.*	0	0	0	0	2	2
		Tinodes sp.	0	1	0	0	0	1
	Xiphocentropodidae	*Xiphocentron sp.*	0	1	0	0	2	3
	Calamoceratidae	*Anisocentropus sp.*	2	1	1	1	4	9
	Odontoceridae	*Psilotreta sp.*	1	1	1	2	5	10
	Philopotamidae	*Dolophilodes sp.*	0	1	2	49	74	126
	Stenopsychidae	*Stenopsyche sp.*	0	0	0	8	20	28
	Brachycenridae	*Brachycentrus sp.*	2	2	2	12	12	30
	Lepidostomatidae	*Goerodes sp.*	0	0	0	3	13	16
		Neoseverinla sp.	1	0	0	0	5	6
Total			344	613	405	763	1065	3190
Mean±SE			18.11± 5.87[a]	32.26± 4.29[ab]	21.32± 5.76[a]	40.16± 5.44[b]	56.05± 4.25[c]	167.89± 5.41
ODONATA	**Gomphidae**	*Lamelligomphus sp.*	48	288	60	79	193	668
		Leptogomphus sp.	23	105	20	55	77	280

		Gomphidia sp.	3	6	14	1	4	28
		Paragomphus sp.	52	56	36	16	10	170
		Sleboldius sp.	5	11	6	0	25	47
		Heliogomphus sp.	7	9	7	12	8	43
		Labrogomphus sp.	7	3	1	0	1	12
		Ophiogomphus sp.	4	1	1	0	8	14
		Sinictinogomphus sp.	0	2	2	0	2	6
		Sinogomphus sp.	3	2	2	0	0	7
		Gastrogompus sp.	4	2	1	0	0	7
		Stylogomphus sp.	0	0	3	0	4	7
	Cordullidae	*Cordulia sp.*	6	5	20	1	0	32
		Epitheca sp.	21	3	59	4	3	90
		Somatochlora sp.	0	0	1	1	0	2
	Libellulidae	*Libellula sp.*	36	10	48	7	7	108
		Nannophya sp.	27	1	35	5	0	68
		Acisoma sp.	12	2	22	3	2	41
		Brachythermis sp.	14	0	28	1	0	43
		Deielia sp.	4	1	9	0	0	14
		Trithemis sp.	13	0	10	0	0	23
		Diplacodes sp.	23	2	22	2	0	49
	Macromidae	*Macromia sp.*	4	15	24	7	2	52
	Coenagrionidae	*Coenagrion sp.*	7	11	14	3	4	39
	Platycnemididae	*Platycnemis sp.*	17	0	27	2	7	53
		Copera sp.	7	2	35	5	7	56
	Platystictidae	*Drepanosticta sp.*	7	11	14	3	26	61
	Protoneuridae	*Prodasineura sp.*	44	11	21	5	6	87
	Lestidae	*Indolestes sp.*	6	2	6	1	5	20
		Lestes sp.	1	1	1	1	1	5
	Chlorolestidae	*Sinolestes sp.*	14	17	44	28	46	149
		Megalestes sp.	17	12	19	13	17	78
	Calopterygidae	*Calopteryx sp.*	123	56	28	19	8	234
		Neurobasis sp.	8	13	28	15	1	65
		Matrona sp.	3	1	0	1	1	6
	Chlorocyphidae	*Libellago sp.*	0	4	4	0	2	10
		Rhinocypta sp.	3	0	7	6	8	24
	Euphaidae	*Bayadera sp.*	29	41	69	35	98	272
		Anisopleura sp.	15	15	25	9	46	110
Total			617	721	773	340	629	3080
Mean±SE			15.82± 4.47[b]	18.49± 4.34[b]	19.82± 5.52[b]	8.72± 2.93[a]	16.13± 3.5[b]	78.97± 2.97
HEMIPTERA	**Aphelocheridae**	*Aphelocherius sp.*	3	2	2	41	8	56
	Nepidae	*Ranatra sp.*	5	5	2	1	0	13
		Nepa sp.	1	1	0	0	0	2
		Laccotrephes sp.	2	1	1	1	0	5
	Belostomatidae	*Lethocerus sp.*	97	1	8	0	2	108
		Diplonychus sp.	33	1	3	0	1	38
	Naucoridae	*Naucoris sp.*	100	25	200	51	21	397
		Ctenepocoris sp.	141	65	207	85	63	561
		Heleocoris sp.	11	6	11	13	8	49
	Notonectidia	*Notonecta sp.*	2	1	0	0	0	3
	Pleidae	*Paraplea sp.*	1	2	0	2	3	8
	Vellidae	*Rhagovelia sp.*	24	58	14	23	1	120
		Angilia sp.	3	4	0	12	0	19

	Gerridae	*Rhagadotarsus sp.*	26	40	56	43	10	175
		Gerris sp.	3	3	2	0	0	8
	Hydrometridae	*Hydrometra sp.*	2	0	0	0	0	2
Total			454	215	506	272	117	1564
Mean±SE			28.38± 3.69^{a}	13.44± 2.13^{ab}	31.63± 191^{a}	17± 2.59^{b}	7.31± 2.62^{a}	97.75± 2.51
COLEOPTERA	**Hydroscaphidae**	*Hydroscapha sp.*	2	4	3	1	0	10
	Dytiscidae	*Dytiscus sp.*	25	6	7	7	4	49
		Laccophilus sp.	131	48	40	21	3	243
		Copelatus sp.	0	1	0	1	0	2
		Cybister sp.	1	1	2	0	0	4
	Gyrinidae	*Dinectus sp.*	5	15	1	7	3	31
	Amphizoidae	*Amphizoa sp.*	0	7	3	1	5	16
	Hydraenidae	*Limnebius sp.*	28	45	15	25	4	117
	Elmidae	*Stenelmis sp.*	5	22	5	26	37	95
		Potamophilus sp.	0	0	0	0	5	5
	Dryopidae	*Elmomorphus sp.*	1	3	3	15	16	38
	Hydrophilidae	*Helochares sp.*	14	1	33	15	2	65
		Hydrophilus sp.	0	1	0	0	0	1
		Berosus sp.	0	1	0	0	0	1
		Tropisternus sp.	0	2	2	8	7	19
		Amphiops sp.	1	1	3	2	1	8
	Psephinidae	*Mataeopsephus sp.*	3	3	15	73	115	209
		Eubrianax sp.	0	0	2	13	23	38
	Sperchidae	*Spercheus sp.*	1	0	4	0	0	5
	Scritidae	*Cyphon sp.*	0	0	2	0	0	2
Total			217	161	140	215	225	958
Mean±SE			10.85± 1.98^{ab}	8.05± 2.13^{ab}	7.00± 1.91^{a}	10.75± 2.59^{ab}	11.25± 2.62^{b}	47.9± 2.51
MEGALOPTERA	**Corydalidae**	*Protothermes sp.*	0	0	1	2	2	5
		Neochauliodes sp.	2	1	2	39	51	95
Total			2	1	3	41	53	100
Mean±SE			1±0.08^{a}	0.05± 0.06^{a}	1.5± 0.127^{a}	20.5± 0.65^{b}	26.5± 0.39^{b}	50±0.35
LEPIDOPTERA	**Pyralidae**	*Ostrinia sp.*	3	1	1	6	8	19
Total			3	1	1	6	8	19
Mean±SE			3±0^{ab}	1±0^{a}	1±0^{a}	6±0^{bc}	8±0^{c}	19±0
DIPTERA	**Tipulidae**	*Tipula sp.*	5	1	0	3	7	16
		Hexatrona sp.	17	21	9	46	48	141
	Ceratopogonidae	*Dasyheleina sp.*	7	14	0	1	0	22
		Bezzia sp.	1	0	15	13	12	41
	Chironomidae	*Chironomus sp.*	2	3	11	3	4	23
	Simulidae	*Simulium sp.*	4	8	21	12	6	51
	Tabanidae	*Tabanus sp.*	8	2	14	9	0	33
	Athericidae	*Atherix sp.*	3	3	4	77	10	97
		Atrichops sp.	1	1	1	0	1	4
	Ephydridae	*Ephydra sp.*	2	2	1	2	5	12
Total			50	55	76	166	93	440
Mean±SE			5±0.50^{a}	5.5± 0.58^{a}	7.6± 1.193^{a}	16.6± 1.86^{b}	9.3± 0.71^{a}	44±1.21
Grand Total			2449	2517	2235	2754	3555	13510

Note: a,b,c are the homogenous groups between sites by Duncans multiple comparison range test

Table 2. Biological indices of aquatic insects

Indices	Site 1	Site 2	Site 3	Site 4	Site 5	Total
Shannon Weiner Diversity Index	3.20	3.16	2.98	3.26	3.27	3.82
Simpson Dominance Index	0.93	0.93	0.92	0.94	0.94	0.96
Margalef's Richness Index	8.11	8.21	7.24	8.44	8.90	13.04

sp. The least abundant family among Ephemeroptera was Tricorythidae with only one genus *Tricorythus sp.* and it was present only in site 5. In the order Plecoptera only one family was obtained, Perlidae. Among Perlidae most abundant genus was *Neoperla sp.* and least abundant was *Tetropina sp.* Numerically, the third abundant order was Odonata. From this the most abundant family was Gomphidae with twelve different genera and the least abundant family was Lestidae. In the order Hemiptera the most abundant family was Naucoridae with three different genera and the least dominant family was Hydrometridae and this family was present only in site 1. From the order Coleoptera the most abundant family was Dytiscidae with four different genera and the least abundant family was Scritidae and it was present only in site 3. Megaloptera and Lepidoptera are the least abundant orders and were represented with only one family each. In Diptera the most abundant family was Tipulidae and is found to be maximum in site 5 and minimum in site 3. The least abundant family was Ephydridae.

Organization of functional feeding groups and habit categorizations

The major feeding groups are collector- gatherers, collector- filters, predators, scrapers and shredders. The proportion of each functional feeding category is presented in fig.1. In all sites predators were the most dominant functional feeding groups and shredders are the least abundant feeding group.

The main habit categories are clingers, sprawlers, swimmers, skaters, climbers and burrowers. The proportional abundance of habit categories of aquatic insects were represented in fig.2. Clingers were dominant habit at all the sites and skaters were the least dominant habit categorization.

Biological indices

The biological indices of aquatic insects at five sites were represented in table 2. Shannon-Weiner diversity index for five sites were ranged from 2.98 to 3.27 and the maximum value was reported from site 5 and the minimum from site 3. Shannon-Weiner diversity of the entire stream was 3.82. The Simpson dominance index value fluctuated from 0.92 to 0.94 and the highest value was reported in sites 4 and 5 and the lowest value was in site 3. The overall value was 0.96. The Margalef's richness index showed comparatively low value in site 3 (7.24) and high in site 5 (8.90) and 13.04 was the value of the entire stream. The statistical analysis of the diversity indices of the five sites reveled that Shannon Weiner diversity indices shows 1% significant variation between sites while Margalef's richness indices shows 5% significant variation. Simpson Dominance indices don't show significant variation.

DISCUSSION

Aquatic biodiversity is one of the most essential characteristics of aquatic ecosystem for maintaining its stability (Vinson and Hawkins, 1998; Sharma *et al.,* 2004). Biodiversity loss in freshwater ecosystems is an increasing phenomenon, mainly due to human activities (Abell, 2002). Aquatic habitats particularly free flowing tropical Asian streams with acceptable water quality and substrate conditions harbour diverse macro invertebrate

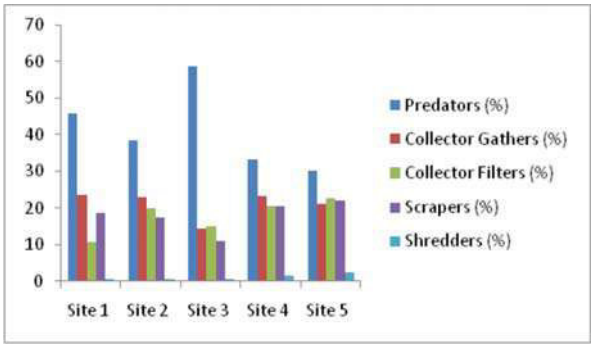

Fig. 1: Proportional abundance of functional feeding groups

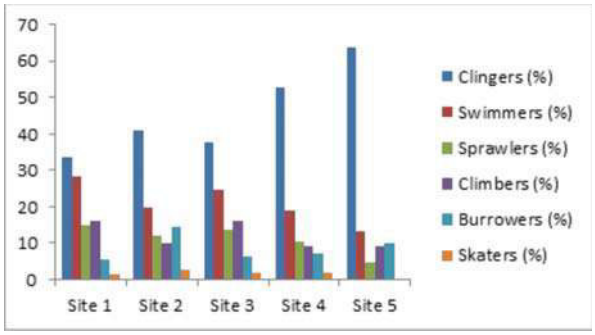

Fig. 2: Proportional abundance of habit categories of insects

communities in which there are a reasonably balanced distribution of species among the total number of individuals present.

In our study, 9 orders comprising 61 families, 125 genera and 13,510 individuals of aquatic insects were collected and identified. Trichoptera was numerically the most abundant order in our study. The results support the findings of Sivaramakrishnan *et al*. (2000). They reported that Trichoptera was the most popular order of aquatic insects in the streams of Western Ghats. According to Dinakaran and Anbalagan (2008) *Hydropsyche sp*. (Hydropsychidae) was the most widely distributed genus in the Western Ghats. In our study also Hydropsyche was the most abundant genus in all the collection sites. Ephemeroptera is one of the intolerant groups of insects which are considered as an indicator of water quality because of its presence in both the polluted and unpolluted reaches of the aquatic body. The genera *Baetis sp*. and *Caenis sp*. from earlier studies have been reported to be tolerant to organic pollution (Menetrey *et al.,* 2008; Abhijna *et al.,* 2012). The genus *Thalerosphyrus sp.* belonging to the Heptagenidae family was found to be intolerant to pollution (Abhijna *et al.,* 2012). In our study *Thalerosphyrus sp.* was abundant in site 5 and absent in site 3. This is because of the poor water quality of site 3 compared to that of other sites.

The order Plecoptera is one of the most pollution sensitive aquatic insect orders. In our study only one family (Perlidae) of Plecoptera were obtained and the same results were obtained by other studies in the streams of Western Ghats region (Anbalagan *et al.,* 2004; Dinakaran and Anbalagan, 2007; Balachandran *et al*., 2012 and Rathinakumar *et al*., 2014). According to Fore *et al.* (1996) and Maxted *et al.* (2000) the order Plecoptera is considered highly sensitive to environmental degradation. In our study maximum number of Plecoptera was reported in site 5 and minimum number was in the site 3, this result clearly indicates the condition of water body. In our study 13 families and 39 genera of Odonates were obtained and it is the 3rd abundant order. Odonata population can be indicative of the richness of other invertebrates and macrophytes (Bried and Ervin, 2005). The sub order Anisoptera (dragonflies) were abundant than that of Zygoptera (damselflies) in all the selected sites in Kallar during the study period. Same result was obtained in other studies from the Western Ghats such as Anbalagan *et al.* (2004) and Balachandran *et al.* (2012).This might be due to their high dispersal ability (Corbet, 1999, Lawler, 2001; Kadoya *et al.,* 2004) and their adaptability to wide range of habitats (Suhling *et al.,* 2004, 2005). Zygoptera would be more affected by environmental characteristics and space than Anisoptera, for being more habitat dependent (Corbet, 1999) and having less dispersal ability (Weir, 1974). The presence of Coleopteran in an aquatic system along with other less tolerant species such as Ephemeroptera, Plecoptera, Trichoptera and Odonata have been observed to reflect clean water conditions (Miserendino and Pizzolon, 2003; Adakole and Annune, 2003). Dytiscidae family generally inhabits leaf of bottom macrophytes of the clean fresh water and is predaceous in nature. Hydrophylidae family in the contrary, are water

scavenger beetles and generally occur in shallower regions of the wetland with abundant macrophytes particularly emergent ones and feed mainly on detritus algae and decaying vegetative matter (Khan and Ghosh, 2001). Chironomidae are widely considered tolerant to organic pollution. Stuijfzand *et al.* (2000) claim the success of this group is better attributed to utilizing organic food sources, rather than tolerance to pollution. Still, it is known that some genera are intolerant to organic pollution (Raunio *et al.,* 2007). According to Yule (2004) Chironomidae is probably the most diverse and abundant group of all stream macroinvertibrates. The standing and slow flowing streams and muddy or sandy areas, with fine sediment particles are known to support higher diversity and abundance of Chironomidae (Yule, 2004). The dominant group in Kallar was predators, and collectors and shredders were the least dominant groups. Collector filters comprised most of the functional feeding group in distribution and can be explained by the most abundant taxa which could be due to their great capacity of wide distribution (Morse *et al.,* 1984). The proportion of collector gatherers highlighted the presence of considerable amount of fine particulate organic matter in the study area (Lemly and Hilderbrand, 2000). The preponderance of collectors in tropical streams may be due to the fact that leaves are decomposed to detritus particles by the microbial community in matter of days leaving little for shredder to feed (Burton and Sivaramakrishnan, 1993). The results of the study showed that the Shannon-Weiner diversity index values ranged from 2.98 (site 3) to 3.27 (site 5). Sharma *et al. (*2008) studied the diversity of aquatic insects in Chandrabhaga River and they reported that the value of Shannon Weiner diversity index ranged from 2.54 to 3.86 and the present results are also in this range. The Simpson dominance index values ranged from 0.92 (site 3) to 0.94 (site 4 and site 5). According to Thakur *et al*. (2013), the lower values indicate comparatively less evenly distributed communities in those sites. Margalef's richness index values shows variation between sites. The highest value of 8.90 was reported in site 5 and the lowest value of 7.24 in site 3. Kocatas (1992) reported that the fall in the value of Margalef's index shows a rise in the level of pollution. The abundance and diversity of aquatic insects in the Kallar stream and its tributaries were found to be highest in site 5 followed by site 4, site 2, site1 and site 3 respectively. In addition to that the most pollution sensitive organisms are highest in site 5 and lowest in site 3 and this clearly indicates the quality of the water body. In the tributaries many anthropogenic activities are taking place and these factors have direct and indirect impact on the diversity of aquatic insects. The conservation and management of the stream is very important for proper functioning of the ecosystem. The present data can be used for monitoring and upkeep of streams of Western Ghats.

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Review of *Semaranga* Becker (Diptera: Chloropidae: Chloropinae) with description of a new species from India

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ABSTRACT: *Semaranga* Becker is reviewed and a second species, *S. subtriangularis* Cherian sp. n. is described from India. © 2016 Association for Advancement of Entomology

KEYWORDS: Chloropidae, Mepachymerini, *Semaranga subtriangularis* Cherian sp. n., India

INTRODUCTION

Semaranga Becker is a small genus known by the type species *S. dorsocentralis* Becker. It is distributed in the Afrotropical and Oriental Regions, including India. Andersson (1977) in his revisionary work on Chloropidae of the world placed the genera *Semaranga* and *Elachiptereicus* Becker under the *Semaranga* genus group proposed by him because of the similarities between the two genera pointed out also earlier by Sabrosky (1951). Later Nartshuk (1983) erected the tribe Mepachymerini and placed the above two genera along with three more namely, *Centorisoma* Becker, *Mepachymerus* Speiser and *Steleocerellus* Frey under it because of some characters they have in common. These tribal placements are followed today. *Semaranga* is unique in the subfamily Chloropinae in possessing three pairs of *dc* bristles on scutum in place of one pair found in all other genera of the subfamily.

While studying the genus *Semaranga* two groups of specimens were observed, one representing true *S. dorsocentralis* species and another, a related but different species. The original description of *dorsocentralis* by Becker was silent on some important characters.The study of the detailed redescription of the species by Andersson (1977) and later by Kanmiya (1983) indicated that the former dealt with true specimens of *dorsocentralis* while Kanmiya based his description probably on two groups of specimens, one representing true *dorsocentralis* and the other a different species as revealed by discrepancies in the descriptions of body characters and diagrams of male genitalia. A new species is described here, its differences with *dorsocentralis* are stated, species limits are drawn and a key to both the species is given.

The type specimens are retained at present in the collections of the Department of Zoology, University of Kerala, Trivandrum and shall later be deposited in the National Zoological Collections, Western Ghats Regional Centre, Zoological Survey of India, Kozhikode (Calicut), Kerala, India.

Genus *Semaranga* Becker

Semaranga Becker, 1911. *Annales Historico-Naturales Musei Nationalis Hungarici,* 9: 48. Type species : *Semaranga dorsocentralis* Becker. By monotypy.

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Diagnosis: Medium-sized shining flies with three pairs of long straight *dc* bristles, reniform *ant* 3, thickened and pubescent black arista and approximated cross-veins.

Emended characters. Head wider and higher than long; frons projecting beyond anterior margin of eye, weakly convex, shining, nontomentose with a few *fr*; frontal triangle large, glabrous, shiny and reaching anterior margin of frons; *if* proclinate, in a row outside frontal triangle along its margin; face rather flat, sloping, higher than wide with rather indistinct facial carina; antenna yellow; *ant* 2 small, almost as long as or longer than wide*; ant* 3 longer than wide or wider than long, reniform with slightly angulate dorsodistal margin; arista terminal, black, broadly thickened with short, dense black pubescence; gena wider than *ant* 3 with punctate hairs mostly in lower half; vibrissal corner not reaching anterior margin of eye; postgena very well developed; parafacialia not very distinct in profile; eye small, broad oval with oblique long axis and very sparse and fine pubescence; palpi short, cylindrical; proboscis short; head bristles with stout *ovt* and *ivt,* long widely divergent *oc*, short, proclinate and divergent *pv*t and 5-6 short *orb*; scutum moderately convex, longer than wide, glabrous, nontomentose, shining yellow to reddish yellow with deeply brown to partly black longitudinal bands; humeral callus yellow with dark spot; pleura glabrous and shining with rather indistinct or distinct maculae; scutellum with nearly rounded or nearly subtriangular distal margin and weakly convex disc; thoracic bristles well developed but *h* 1 and *pa* 2 absent*; npl* 1+2, subequal to *pa* 1; *dc* 3, long, straight; *as* well developed, a little longer than scutellum; *ss* 1 almost half as long as *as.* wing hyaline with *r-m* and *m-m cross*-veins strongly approximated; distance between cross-veins less than length of *m-m;* R_{4+5} and M_{1+2} straight but divergent; haltere yellow; legs slender and elongated; tibial organ long and narrow; abdomen usually suboval, finely tomentose with dark hairs; male genitalia elongate and geniculate; surstylus attached to anteroventral aspect of epandrium; pregonites not developed; postgonites narrowly elongate with a pair of stout long to very long black bristles near its middle; basiphallus narrowly elongate; distiphallus bifid at apex; ovipositor rather short and stout.

Distribution: Oriental and Afrotropical Regions

Remarks: *Semaranga* shows close affinities to members of *Elachiptereicus* Becker (Cherian *et al*., 2014) in the nature and development of head, antenna, wing with approximated *r-m* and *m-m* cross-veins and general nature of male genitalia as emphasized by earlier authors, including Sabrosky (1951), Cherian *et al*. (2014) and others. However *Semaranga* differs from *Elachiptereicus* chiefly in the former having 3 pairs of well developed *dc* bristles, an unusual feature in the subfamilies Chloropinae, Rhodesiellinae and Oscinellinae expect for *Tricimbomyia* Cherian (1989) under Oscinellinae in which 2 pairs of *dc* bristles are present. Hence *Semarang*a is considered a distinct genus as recognized by earlier workers including Nartshuk (1983) who placed it under the tribe Mepachymerini Nartshuk.

This genus is hitherto known by the type species *S. dorsocentralis* Becker which is widely distributed in the Afrotropical and Oriental Regions, including India. It is apparent from the descriptions of *S. dorsocentralis* by earlier workers like Andersson (1977) and especially Kanmiya (1983) and a few others that their description of this species was based on two distinct species, one representing true *dorsocentralis* and the other a different species. According to Kanmiya (1983*),* third segment of arista is 4x as long as the second in *dorsocentralis* but in true *dorsocentralis* and the new species described below*,* 3rd aristal segment is at most 2.2x as long as the second. Kanmiya either might have erred in describing this character, which does not normally happen with his descriptions or else a different species was involved. However specimens studied by Kanmiya are not readily available for verification at present.

Key to species of *Semaranga*

ant 3, 1.3x as wide as long; *ant* 2 about 0.9x as

long as wide; facial carina rather indistinct; scutellum nearly rounded at apex*; as* rather widely separated at base, distance between bases of *as* and *ss* 1 much less than that between bases of *as;* proportions of costal sectors 2 to 4 in the ratio 22:20:11; ……*dorsocentralis* Becker

ant 3, 1.2x as long as wide; *ant* 2, 2x as long as wide; face a little raised medially; scutellum nearly subtriangular at apex*; as* not very widely separated at base, distance between bases of *as* only a trifle more than that between bases of *as* and *ss* 1; second costal sector 1.36 to 1.5x as long as third sector. ……*subtriangularis*,Cherian sp. n.

***Semaranga dorsocentralis* Becker**
(Pl. 1, Figs. 1-4)

Semaranga dorsocentralis Becker (1911): 48. Type localities: Indonesia: Semarang; India: Bombay.

Male and female (Pl.1): Head predominantly yellow to orange yellow, higher than long, length, height and width ratio 8:10:12; frons projecting beyond anterior eye margin, about 1.4x as long as wide and nearly 0.47x as wide as head, yellow to yellowish brown and with a few black *fr* mostly in anterior half; frontal triangle nearly as wide as frons at vertex, glabrous, shiny yellow to orange yellow, in some specimens with deep brown tinge at apex and area immediately behind, reaching anterior margin of frons and ending with pointed apex; face yellow to yellowish brown, rather flat, sloping, higher than long but in some specimens midlongitudinal area along about two-thirds length of face between bases of antennae slightly raised and hence with concave sides and a little raised epistomal margin; antenna yellow but in some specimens basal segments deeply brownish; *ant* 2 almost as long as wide; *ant* 3 reniform, about 1.3x as wide as long, narrowly darkened along dorsodistal margin; arista at apex of *ant* 3, black broadly thickened with very dense short, black hairs; first basal segment of arista as long as wide, second segment about 2x as long as wide, third segment about 1.6x as long as combined length of basal segments and 2.3x as long as second, though according to Kanmiya, third segment is 4x as long as the second; eye small, broad oval with oblique long axis and very sparse, minute pubescence; gena very broad, strongly widened in the area of postgena, width in the middle about 1.3x that of *ant* 3, distinctly rugose with slender, punctate hairs mostly in lower half, in most specimens yellow but a few with dark tinge; vibrissal corner almost a right angle, not reaching anterior margin of frons; cephalic bristles as described for the genus; scutum a little narrower than head and about 1.1x as long as wide, moderately convex, a little flattened posteriorly, smooth, not tomentose, shiny yellow with three dark brown to black broad longitudinal bands of which median commences from anterior margin and in most specimens tapers off a little beyond middle of scutum posteriorly and each submedian band commences from level of lower margin of humeral callus and extends whole length of scutum; besides the three longitudinal bands lateral to each submedian one linear to a little more developed oblong black macula is present which often partly merges with the submedian band; in some specimens median band is largely discoloured, appearing reddish brown to reddish yellow; scutal hairs rather scattered, short pale brown; pleura pale, glabrous, in most specimens with variously developed reddish brown to brown maculae on part of *kepst;* meron and rarely on *anepm*; scutellum about 1.4x as wide as long, shiny yellow with infuscated laterobasal corners as a continuation of the infuscation of submedian dark bands on scutum, with rounded oval distal margin and weakly to distinctly convex disc bearing a few short pale brown hairs; thoracic bristles black, well developed, as described for the genus*; dc* 3, straight much longer than *npl*, of these anterior most is presutural and the rest postsutural in position*; as* straight, as long as scutellum; ss 1 less than half of *as;* distance between bases of *ss* and *as* much less than that between bases of *as;* wing hyaline with brown veins and hairs; proportions of costal sectors 2 to 4 in the ratio 22:20:11; last section of M_{1+2} evanescent; *r-m* cross-vein far distad of middle of discal cell, opposite 0.82 of its length; distance

between *r-m* and *m-m* shorter than length of *m-m*; terminal sectors of R_{4+5} and M_{1+2} divergent; anal field slightly receding; haltere yellow; legs slender with short black hairs, almost entitrely yellow with only the last tarsal segment of all legs infuscated but in older specimens variously developed brown tinge is discernable on coxa, some femora, tibiae and some distal tarsal segments of fore leg; tibial organ long and narrow; abdomen predominantly yellow but in some specimens some segments with dark tinge, longer than wide and wider than thorax, subshiny, finely grey tomentose with short dark hairs. Female cerci relatively short with a few fine hairs; male genitalia (Figs. 1-4) as described for the genus.

Length: Male - 2.2 - 2.5 mm: wing 2.1 - 2.3 mm. Female - 2.4 - 2.8 mm; wing 2.3 - 2.7 mm.

Specimens studied: 2 ♂, 2 ♀; Nicobar Is., Camerota, 40.0319° N, 15.3751° E 6. x. 1972, Coll. P.T. Cherian; 1 ♀ (head broken off), Meghalaya: Shillong; Mawphlang. 6. ix. 1975, Coll. N. Muraleedharan; 1 female; Meghalaya: Shillong. 9. ix. 1975, Coll. N. Muraleedharan; 2 ♂, 8 ♀; Meghalaya: Cherrapunji; 5 .v. 1979, Coll. G.K. Srivastava.

Remarks: *S. dorsocentralis* is very widely distributed in the Oreintal and Afrotropical Regions and is the only species of *Semaranga* known. Because of the discrepancies in the descriptions and differences in the diagrams of the genitalia of *dorsocentrali*s by earlier workers like Andersson (1977) and Kanmiya (1983), it is evident that Kanmiya had dealt with two distinct species, one representing true *dorsocentralis* and the other a different species. Based on the present study of specimens from diverse demes and their male genitalia, it is apparent that Andersson's description was based on the study of true specimens of *dorsocentralis* whereas that by Kanmiya was probably based on some specimens of *dorsocentralis* and also others representing a different species. The differences between the two are given in the key to species and under remarks that follows the description of the new species.

Distribution: China: Kiangsi, Yunnan, India: Meghalaya, Maharashtra, Nicobar Is, W. Bengal; Indonesia: Java; Philippines: Luzon; Russia: Maritime territory; widely distributed in Africa; Japan: Honshu, Kyushu, Amami and Ishigaki Islands, Hawai.

***Semaranga subtriangularis* Cherian sp.n.**

LSID urn:lsid:zoobank.org:act:8C8C348B-9F1D-43DD-B2B6-6F94A7621FAC

(Pls. 2-4, Figs. 5-6)

Male [(Pl. 2) and female: Head (Pl. 3) is Predominantly yellow, higher than long, length, height and width ratio 16:19:25. Frons projecting a little beyond anterior margin of eye but less so than in *dorsocentralis*, 1.2x as long as wide and 0.52x as wide as head at vertex, yellow to yellowish brown, very finely tomentose with a few well developed black *fr;* frontal triangle nearly as wide at vertex as frons, large, glabrous, shiny yellow to orange yellow, reaching anterior margin of frons and ending with pointed apex. Face yellow to yellowish brown with dark tinge around epistomal margin in some specimens, sloping, higher than long, mid longitudinal area a little raised up to epistomal margin, giving the impression of a distinct facial carina, especially in some specimens. Basal antennal segment hidden by projecting frons; *an*t 2 yellow but with distinct dark tinge in some specimens, 2x as long as wide unlike in *dorsocentralis* in which it is only almost as wide as long*; ant* 3 reniform, 1.2x as long as wide, yellow but infuscated along dorsodistal margin; arista at apex of *ant* 3, black, broadly thickened with short, very dense black hairs; proportions of lengths of three flagellar segments in the ratio 2:5:11; second flagellar segment a trifle more than 2x as long as wide. Gena wide, very widened at area of postgena, width in middle 1.3x that of *ant* 3, distinctly rugose as in *dorsocentralis* with slender, punctate hairs mostly in lower half, yellow to brownish yellow; vibrissal corner almost a right angle; parafacialia narrow, often not visible in profile. Proboscis short, yellow but a little infuscated in some specimens; palpi cylindrical, yellow but rarely appearing infuscated because of black hairs. Eye relatively small, broad oval with oblique long axis and very minute, sparse

Plate 1. ***Semaranga dorsocetralis*** **Becker, Female fly**

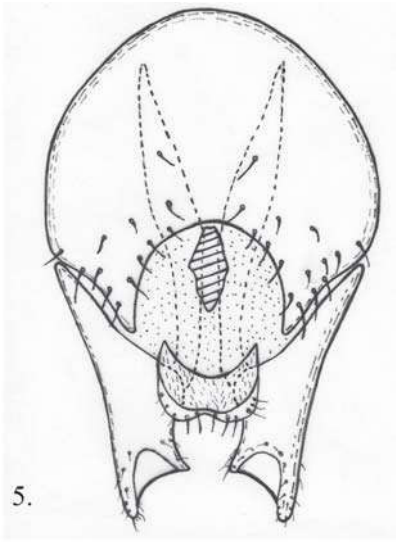
Plate 2-4. ***Semaranga dorsocetralis*** **sp.no. 2. Male fly, 2. I lead, dorsal view, 4. Scutellum.**

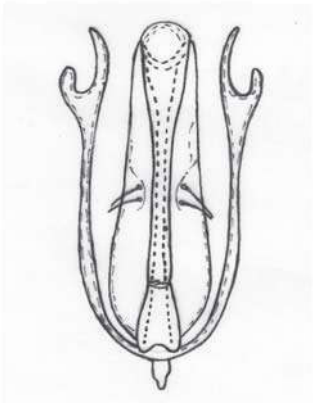
pubescence. Head bristles as in *dorsocentralis* with well developed *ovt* and *ivt*, long, proclinate and divergent *oc*, short, slender, proclinate and slightly divergent *pvt*, 5-6 *orb* and 5-6 well developed, proclinate *if* along margin of frontal triangle mostly in anterior half.

Thorax: Scutum a little narrower than head and as wide as long, moderately convex but less so posteriorly, smooth, not tomentose, shiny yellow with three reddish brown to dark brown, broad longitudinal bands as in *dorsocentralis* but in some specimens including the holotype, median band is very faint and almost indistinct and in all specimens it commences from anterior margin of scutum, is abbreviated posteriorly and fades off around middle of scutum and each submedian is often divided at around transverse suture and appears on each side as two distinct bands below transverse suture; humeral callus yellow with dark spot medially; scutal hairs scattered, pale brown; pleura glabrous, shiny yellow with reddish brown to a little infuscated large macula on *meron* and part of *kepst* and more faint smaller maculae on *anepm* but in some specimens the maculae are rather indistinct and appear as glabrous and shiny patches only. Scutellum (Pl. 4) nearly subtriangular, 1.35x as wide as long, with less convex and almost flattened yellow disc than in *dorsocentralis* which is often with brown to dark brown infuscation at laterobasal corners which extends a little more along lateral margins. Thoracic bristles well developed; *npl* 1+2, subequal and equal to *pa* 1; *dc* 3, straight, much longer than *npl*, sequentially posterior ones becoming longer and stouter; distance between bases of posterior most *dc* much more than that between those of *dc* 1 and *dc* 2 as in *dorsocentralis*; *as* 1.2x as long as scutellum; *ss* 1, 0.55x the *as*; bases of *as* nearer to each other than in *dorsocentralis* and only a trifle more than that between bases of *as* and *ss* 1.

Wing: Hyaline 2.58x as long as wide with yellowish brown to brown veins and brown hairs; proportions of costal sectors 2 to 4 in the ratio 19:14:9 to 33:22:15; *r-m* cross vein far distad of middle of discal cell, opposite 0.85 of its length; length of *m-m* 1.5x the distance between *r-m* and *m-m*; terminal sector of M_{1+2} evanescent and gradually diverging from that of R_{4+5}; anal corners slightly receding. Haltere yellow.

Legs: Slender with short yellow and dark hairs; coxae, femora and tibiae yellow with brown tinge in some areas under certain angles of illumination; tarsi yellow except for last tarsus of all legs; in some





Figs. 5-6: ***Semaranga subtriangularis*** **sp.n.**
5. Epandrium, posterior view
6. Phallic complex, ventral view

specimens most of fore tarsi appear a little infuscated under some angles of illumination; tibial organ long and narrow as in *dorsocentralis.*

Abdomen: Much longer than wide, predominantly blackish brown but rarely appearing more yellowish, subshiny, finely tomentose with a few well developed slender dark hairs, ovipositor short rather stout. Male genitalia (Figs 5-6): surstylus with median depression on distal margin; mesolobus large, medially concave distally with well developed hairs; hypandrium long and narrow; pregonite absent; postgonite more narrowly elongate than in *dorsocentralis* with a pair of stout black setae medially which are relatively shorter than in *dorsocentralis*; basiphallua and phallopodeme narrowly elongate with a slightly sclerotized plate at base of distiphallus.

Length: Male 2.2 - 2.7 mm; wing 2.0 - 2.4 mm
Female 2.3 - 3.4 mm; wing 2.3 - 2.7 mm

Holotype: ♂, Kerala: Trivandrum 8.5241° N, 76.9366° E Kariavattom. 25 m. 6.xi.2006. Coll. Jyothi Tilak. *Paratypes*: 1 ♀, Tamil Nadu: Palani Hills, 10.2000° N, 77.5000° E 27. iv. 1989. Coll. P.T. Cherian; 1 ♀(?), Karnataka: Bodipode: Biligiri 11.9956° N, 77.1428° E. WLS. 18 .iii. 1999. Coll. S. Krishnan; 1 ♂, Kerala: Trivandrum., Kariavattom. 25 m. 25. x. 2004, Coll. J. Jasmin; 1 ♀, Kerala: Wayanad Dist., Kabanigiri. 11.8574° N, 76.1812° E 750 m. 7 .i. 2006. Coll. A.K.Shinimol; 2 ♀, Kerala: Trivandrum Dist., Kariavattom. 25 m. 6.xi.2006. Coll. Jyothi Tilak; 1 ♀, Kerala: Trivandrum Dist., Veli. 10m. 2.xii.2007. Coll. Jyothy Tilak.

Remarks: *S. subtriangularis* shows close affinities to *dorsocentralis* Becker but in the former *ant* 3 is longer than wide, *ant* 2 is 2x as long as wide, scutellum is nearly subtriangular with more flattened disc, *as* are less widely separated at base and second sector of costa is 1.36 to 1.5x as long as third sector. But in *dorsocentralis ant* 3 is wider than long, *ant* 2 is not longer than wide, scutellum is with rounded oval distal margin and more convex disc, *as* are more widely separated at base and second sector of costa is only a trifle longer (11:10) than third sector. Besides, both species differ in relative development of male genitalia as shown in the figures.

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ABBREVIATIONS

anepm - anepimeron, *anepst* - anepisternum, *ant* 2 - second antennal segment, *ant* 3 - third antennal segment, *as* - apical scutellar bristle, *dc* - dorsocentral bristle, *fr* - frontal hair, *h* - humeral bristle, *if* - interfrontal bristle, *ivt* - inner vertical bristle, *kepst* - katepisternum, *npl* - notopleural bristle, *oc* - ocellar bristle, *orb* - frontoorbital bristle, *ovt* - outer vertical bristle, *pa* - postalar bristle, *pvt* - postvertical bristle, *ss* - scutellar bristle, $R_{2+}3$- radius $_{2+3}$, R_{4+5} - radius $_{4+5}$, M_{1+2} - median vein$_{1+2}$.

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New record of scales and mealybugs (Hemiptera: Coccoidea) infesting sandalwood (*Santalum album* Linn.) in agroforestry conditions

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ABSTRACT: Survey conducted on sandalwood, *Santalum album* Linn. growing in agroforestry conditions revealed infestation of 31 species of scales and mealybugs. Of these, seven are new records on *S. album*.

KEY WORDS: Sandalwood, coccids, species of scales and mealybugs

Agroforestry systems are not new to India; traditionally each and every Indian locality has its own types of indigenous agroforestry systems (Dhyani and Handa,2013). Indian sandalwood, *Santalum album* Linn. is emerging as one of the important agroforestry species due to the amendments in the Sandalwood acts in 2001 and 2002, respectively by the Karnataka and Tamil Nadu governments. The Amended Acts clearly states that "every occupant or the holder of the land shall be legally entitled to the sandalwood tree in his land". This is encouraging community and private entrepreneurs to cultivate *S. album* in agroforestry, farm forestry and varied agri-silvi-horticultural and mixed plantation systems (Sundararaj, 2014a). Farmers are growing *S. album* along with other agricultural, horticultural, commercial and other tree species based on their need and choice. Trees like, *Tectona grandis* L.f., *Grevilliea robusta* A. Cunn. ex R. Br., *Azadirachtai ndica* A. Juss, *Tamarindus indica* L., *Melia dubea* Cav., *Simarouba glauca* DC., *Pongamia pinnata* (L.) Pierre, *Pterocarpus santalinus* L.f., *Cassia siamea* L. and *Ailanthus excels* Roxb; horticultural crops like *Anacardium occidentale* L., *Areca catechu* L., *Cocos nucifera* L., *Phyllanthus emblica* L., *Moringa oleifera* Lam, *Citrus reticulata* Blanco, *Punica granatum* L., *Psidium guajava* L., *Carica papaya* L., and *Musa* spp. and agricultural crops like cucurbitaceous vegetables, chillies and lemon grass were found commonly grown with *S. album*. The intercultivation of sandalwood with other plants are commonly preferred than the pure plantations (Sundararaj, 2014b). Surveys were conducted at an interval of once in four months for two years (2014 and 2015) to study the insect pest problems of *S. album* growing outside forest in different agroforestry conditions and the findings related to scales and mealybugs infesting *S. album* is presented in this communication.

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Table 1. Scales and Mealybugs infesting on *S. album* in India

Sl.No	Family	Scientific name	Common name
1.	**I. Coccidae**	*Cardiococcus bivalvata* (Green)	Bivalved scale
2.		*Ceroplastes actiniformis* Green	Coconut wax scale
3.		*Ceroplastes ceriferus*(Fabricius)	The Indian wax scale
4.		*Coccus viridis*(Green) *	Green coffee scale
5.		*Parasaisseti anigra* (Nietner)	Nigra scale/Black bug
6.		*Pulvinaria psidii*Maskell	The green shield scale
7.		*Saissetia coffeae* (Walker)	Hemispherical scale
8.		*Megapulvinaria maxima* (Green)	Neem scale
9.		*Pulvinaria polygonata*Cockerell*	Cottony citrus scale
10.	**II. Diaspididae**	*Abgrallaspis cyanophylli*(Signoret) *	Cyanophyllum scale
11.		*Aonidiella orientalis*(Newstead)	Oriental scale
12.		*Chrysomphalus aonidum*(Linn.)*	Black scale
13.		*Fiorinia fioriniae*TargioniTozzetti	Fiorinia/Avacado scale
14.		*Hemiberlesia lataniae*(Signoret)*	Latania scale
15.		*Ischnaspis longirostris*(Signoret)*	Black line scale
16.	**III. Kerridae**	*Paratachardina lobatalobata* (Chamberlin)	Lobate scale/ pseudo scale
17.		*Paratachardina silvestri*(Mohdihassan)	The pseudolac scale
18.	**IV. Margarodidae**	*Hemaspidoproctus cinereus*(Green)	Giant mealybug
19.		*Perissopneumon phyllanthi*(Green)	-
20.	**V. Monophlebidae**	*Icerya aegyptiaca*(Douglas)	Egyptian mealybug
21.		*I. formicarum*Newstead	-
22.		*I. purchasi*Maskell	Cottony cushion scale
23.		*I. seychellarum*Westwood	Common white mealybug
24.		*Labioproctus poleii*(Green)*	
25.	**VI. Ortheziidae**	*Orthezia insignis*(Browne)	Croton bug
26.	**VII. Pseudococcidae**	*Ferrisi avirgata*(Cockerell)	Striped mealybug
27.		*Nipaecoccus filamentosus*(Cockerell)	Spherical mealybug
28.		*Nipaecoccus viridis*(Newstead)	Coconut mealybug
29.		*Pseudococcus longispinus*(TargioniTozzetti)	Long tailed mealybug
30.		*Rastrococcus iceryoides*(Green)	Mutabilis mealybug
31.		*Lankacoccus ornatus*(Green)	Jasmine mealybug

* new record on *S. album*

The study revealed 31 species of scales and mealybugs under 7 families infesting *S. album* in India (Table 1). Among the 31 species, the infestation of 7 species viz., *Coccus viridis*, *Pulvinaria polygonata*, *Abgrallaspis cyanophylli, Chrysomphalus aonidum*, *Hemiberlesia lataniae, Ischnaspis longirostris* and *Labioproctus poleii* on *S. album* form the new records. The infestation of these scales and mealybugs on *S. album* confirms the earlier reports

(Varshney, 1992 and 2002) of their polyphagous nature. Sundararaj *et al*. (2006) reported the infestation of 23 species of scales and mealybugs and Sundararaj (2011) reported the infestation of Croton bug*, Orthezia insignis* on *S. album,* thus a total of 24 species of scales and mealybugs were earlier known to infest *S. album*. Among the more than 150 insects known to occur on *S. album* in India, the infestation by sucking insects belonging to the family Coccidae is very deleterious as they affect the normal growth and reproduction of sandal plants (Remadevi *et al*., 2005). Often the infestation of *Cardiococcus bivalvata*, *Parasaissetia nigra, Saissetia coffeae, Ceroplastes actiniformis, C. ceriferus* and *Paratachardina silvestri* results in drying of branches causing dieback symptoms and ultimately death in seedlings and trees (Sundararaj *et al*., 2006). The affected flowers wither and fruits dry and fall off prematurely and do not germinate (Sivaramakrishnan *et. al*., 1987). In agroforestry conditions,very often the infestation of *Ca. bivalvata, Ce. actiniformis, Coccus viridis, M. maxima, A. orientalis, I. aegyptiaca* and *Nipaecoccus viridis* were severe resulting in dieback symptoms and death of young trees. Ananthakrishnan (2007) commented that climate change is expected to bring extension in the host range of many pests and diseases and the microclimate of many sucking pests will tent to change, leading to acceleration of their reproductive cycles, resurgence, behaviour and reproductive potential. Hence in the present context of growing *S. album* in agroforestry conditions outside forest a holistic approach, for the better management of economically important coccids is very much required to increase the production of sandalwood in pace with increased area of cultivation.

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Shrot Communicationt. 41413

Population increase of poultry wing louse, *Lipeurus caponis in vivo* condition

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ABSTRACT: Studies regarding the rate of population increase of poultry wing louse *Lipeurus caponis in vivo* condition revealed that initial inoculums of 10 *L. caponis* could produce an average of 318 lice after 90 days in summer (indicating the doubling time to be 18 days) and during winter months it produced 336 lice (the doubling time 22 days). Thus, studies clearly indicated that ischnoceran lice (e.g. *L. caponis*) multiplied population at moderate rate. Summer months are more favorable for population build up of lice.

KEY WORDS: Phthiraptera, poultry lice, *Lipeurus caponis,* population build up

Information regarding the rate of population increase of parasitic insects attracts the attention of parasitologist /biologist and also the veterinarians. Only few workers have made attempts to furnish information on the rate of population increase (*in vivo* condition) of phthirapterans infesting avian hosts. Some clues on the aspects can be derived from the contributions of Glees and Raun (1959), Stockdale and Raun (1960), Brown (1970), Gupta *et al.*, (2007) and Saxena *et al.*, (2007). Information on the rate of population increase of two mammalian ischnocerans has been noted by Murray and Gordon (1969) and Rust (1974). Keeping in view the lacuna prevailing in the field, it was found worthwhile to study the rate of expansion of ischnoceran poultry lice. The present report deals with the rate of population increase of poultry wing louse, *Lipeurus caponis*.

Ten adults of *Lipeurus caponis* were released on the wings of each of the twelve louse free fowls of age 6 months. The aforesaid lice were transferred from lice infested chicken with the help of camel hair brush. The artificially infested fowls were individually housed in wire meshed cages (prevented to come in contact) and provided with poultry feed and water (during April 2013). Two of the artificially infested fowls were subjected to delousing fortnightly by fumigation method. The fowls were placed in large polythene bag containing a wad of cotton wool, soaked in chloroform in such a way that head protruded to allow breathing. The bird was taken out after 15 minutes and feathers manually ruffled over white plastic sheet to recover the lice load. The fowls were further searched to recover the remaining lice load with the help of hand lens fitted with circular light tube. The lice loads so obtained were stored in 70% alcohol and separated stage wise. Lice were identified with the help of information given by Ansari (1943), Same experiment was repeated in November 2013.

As indicated in methodology two fowls were subjected to delousing fortnightly in the months of summer (April 2013 to June 2013). First two fowls deloused after 15 days yielded a total of 32 *L.*

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caponis (4 adults, 28 nymphs). Two fowls deloused after 30 days yielded 50 lice (28 adult, 22 nymphs). Likewise, the number of lice obtained from fowls deloused after 45, 60 and 75 days remained 120 (76 adults, 44 nymphs), 174 (92 adults, 82 nymphs) and 344 lice (120 adults, 224 nymphs). Finally, last two fowls deloused after 90 days yielded 636 lice (280 adults and 356 nymphs). Thus, initial inoculums of 10 *L. caponis* produced on an average of 318 lice after 90 days (Fig.1). Thus, by applying the back roll method the doubling time of the population of *L. caponis* appeared to be 18 days, during summer months.

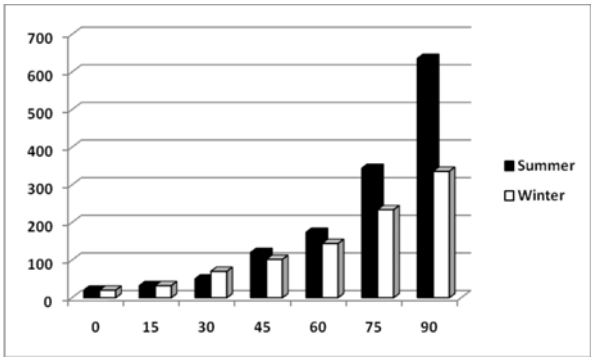

Figure 1. Showing the total number of *Lipeurus caponis* recovered from two fowls (Each inoculated with 10 lice) deloused fortnightly, during 2013

Same experiment was repeated during winter months (November 2013 to January 2014). Two fowls deloused after 15 days were found infested with 32 lice (06 adults, 26 nymphs). The number of lice recovered from fowls deloused after 30, 45, 60 and 75 days yielded 70 (32 adults, 38 nymphs), 102 (54 adults, 48 nymphs), 144 (68 adults, 76 nymphs) and 234 lice (94 adults, 140 nymphs). The last two fowls deloused after 90 days yielded 336 lice (124 adults, 212 nymphs) (Fig.1). Thus, initial inoculums of 10 lice could produced178 lice indicating it's doubling time to be 22 days during winter months. The data obtained from delousing of chickens during summer and winter was tested with the help of χ^2 and the difference was found significant (χ^2=38.9; df =5; p =.05)

There are only few studies relating to rate of population expansion of phthirapteran parasitizing avian host's *in vivo* condition. While recording the economic effects of parasitism of chicken body louse, *Menacanthus stramineus*, Glees and Raun (loc cit.) released 10 lice on each of domestic hens and observed that their numbers increased to 23,063 during a span of 14 weeks. Likewise, while performing similar studies on same louse, Stockdale and Raun (loc cit.) found that 3 adult female could increase up to 12,305 in 16 weeks. However, Brown (loc cit.) released an initial population of 50 chicken body louse (*Menacanthus stramineus*) and found that numbers increased to 1584 in 31 days on debeaked chickens while 50 lice released on beaked (normal) birds could not increase beyond 56 lice. Saxena *et al.* (loc cit.) released an initial population of 14 ischnoceran louse, *Goniocotes gallinae* / bird and found that their population became 1267 in 14 weeks (doubling time 14 days). Likewise, in case of red amandava louse, *Brueelia amandavae* the initial inoculums of 5 lice could build up an average of 60 lice per bird during a span of 75 days. Thus the doubling time of aforesaid louse was computed by (Gupta *et al.* (loc cit.) as 21.5 days. During present studies the doubling time of poultry wing louse, *Lipeurus caponis* appeared to be 18 days (in summers) under *in vivo* conditions in contrast 22 days in winters, indicating that environment plays important role in determining the rate of population expansion of avian lice.

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Short Communication. 41414

Crotonothrips polyalthiae Mound & Nasruddin (Thysanoptera: Tubulifera) – a new record for India

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ABSTRACT: *Crotonothrips polyalthiae* Mound & Nasruddin (2012), a member of phlaeothripid (Insecta: Thysanoptera: Tubulifera) has been recorded for the first time from India, which was erstwhile known only from Malaysia and Indonesia. The diagnostic characters of this species are discussed along with the key to identify other known species of *Crotonothrips*.

KEY WORDS: *Crotonothrips polyalthiae*, new record, Thysanoptera, Tubulifera

The family Phlaeothripidae is the single family in the suborder Tubulifera with maximum number of taxons under the order Thysanoptera. It consists of two subfamilies, Idolothripinae and Phlaeothripinae which are distinguished on the basis of width of maxillary stylet that being broad (>5µm) and band like in the former and they feed exclusively on fungal spores. On the contrary, members of the subfamily Phlaeothripinae comprise a mixed group of individuals of both myco and phytophagous forms with maxillary stylets of 2 or 3µm broad for most of their length (Palmer *et al*., 1989). The family Phlaeothripidae currently includes about 3649 species worldwide (Thripswiki, accessed on 02.06.16), and about 12 per cent of them are known from India. So far 430 species in 143 genera have been reported from India (Tyagi and Kumar, 2016). In a recent survey carried out at Bhubaneshwar, Odisha, a species namely *Crotonothrips polyalthiae* Mound and Nasruddin (Phlaeothripidae: Phlaeothripinae) has been collected and its occurrence in India is reported here for the first time. The details of the collection and diagnostic features are discussed in this article along with the key to identify other known species of the genus *Crotonothrips*.

Diagnostic features of the genus *Crotonothrips* Ananthakrishnan

The genus *Crotonothrips* is characterized by reticulate head and pronotom, fore tarsus with a tooth in both sexes, much reduced mesopraesternum, S2 setae of tergite IX of both sexes about half as long as S1 and tube longer than head with short anal setae. Members of the genus *Crotonothrips* are known to induce plant galls and live within the leaf galls of a wide variety of plants. The genus *Crotonothrips* was erected by Ananthakrishnan in 1967 which comprised of 16

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species as per Thripswiki-accessed on 02.06.2016 (Table -1a and 1b). Among them, 14 species have been originally described from India, while *C. dentifer* from Japan and *C. polyalthiae* from Indonesia & Peninsular Malaysia.

The collected specimens were identified using appropriate keys (Mound & Nasruddin, 2012) and were confirmed by Dr.Mound as *C. polyalthiae* Mound &Nasruddin (2012).The image was photographed using the microscope (Leica stereo zoom Microscope, Leica M 205A).

Material Examined: 11 females, 01.i.2016, leaf galls of *Polyalthia longifolia,* (Family: Annonaceae), Bhubaneshwar (Latitude 20^0 0' 37.3'' N, Longitude $85^0$49' 59'' E), INDIA, Coll. R.R. Rachana. These specimens are deposited with ICAR - National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bangalore, Karnataka, India.

Diagnostic features of the species *Crotonothrips polyalthiaae* Mound and Nasruddin

Body and legs dark brown, fore tarsi and apex of fore tibia yellow; antennal segments I and VII-VIII brown, II yellow at apex, III almost clear yellow but weakly shaded at apex, IV-VI yellow on basal two-thirds, half or third respectively. Fore wing extensively shaded, paler at apex, clear near base around sub-basal setae and with a pale longitudinal line close to posterior margin. Mouth cone pointed,

Table -1a: Setae length of IX abdominal segment of different species of *Crotonothrips**

Thrips species	S1 (µm)	S2 (µm)	S3 (µm)
1. Crotonothrips coorgensis	153	112	153
2. Crotonothrips davidi	91	72	106
3. Crotonothrips dentifer	175	65	**
4. Crotonothrips dissimilis	126	64	126
5. Crotonothrips erraticus	172	148	256
6. Crotonothrips gallarum	160	80	175
7. Crotonothrips longirostris	140	80	148
8. Crotonothrips memecylonicus	143	61	153
9. Crotonothrips mimicus	106	60	160
10. Crotonothrips nagaensis	200	120	240
11. Crotonothrips nelliampathiensis	120	64	160
12. Crotonothrips parvus	107	31	122
13. Crotonothrips polyalthiae	240	60	265

**Data not available. *Data taken from respective species of thrips publication.
S1, S2 and S3 represent setae present on the IX abdominal segment

Table- 1b: Measurements of IX abdominal segment setae of three species

Thrips species	S1 (µm)	S2 (µm)	S3 (µm)
14. Crotonothrips cacharensis	144	140	200
15. Crotonothrips dantahastha	145	143	156
16. Crotonothrips maoensis	212	210	224

extending between fore coxae; mandible restricted to mouth cone. Fore tarsal tooth stout. Mesopraesternum incomplete medially. S2 setae of tergite IX shorter than S1 and S3 (Figure 1).

Fig 1. *Crotonothrips polyalthiae*

Distribution: India: Odisha (new record), Indonesia and Malaysia.

In addition to the above, the authors had the chance to study some of Prof. T. N Ananthakrishnan's collections of *Crotonothrips.* Comparative analysis of those species with our own collections and relevant literature, a detailed key to the species of *Crotonothrips* has been attempted here, as it was lacking as of now.

Key to identify species of the genus *Crotonothrips* Ananthakrishnan (except *C. dantahastha*)

1. Fore-tarsi with well developed tooth in both the sexes. S2 setae of abdominal segment IX always shorter than both S1 and S3 2

   - Fore-tarsi of both sexes without tooth. S2 setae of abdominal segment IX almost subequal to S1 but shorter than S3.....14

2. Mouthcone not broad but slightly narrow and pointed …….....3

   — Mouthcone broadly rounded …… 4

3. Mesopraesternum in complete medially. Antennal segments I, VII, VIII brown, II yellow at apex, III yellow, IV –VIyellow on basal two thirds, half or third respectively..... *polyalthiae* Mound & Nasruddin, 2012

   — Mesopraesternum boat shaped. Antennal segments I – VI golden yellow, VII & VIII brown.....*longirostris* Muraleedharan & Sen, 1981

4. Body distinctly bicolorous; head, all legs yellow; thorax and abdomen brown ..... *memecylonicus* Ananthakrishnan, 1976

   - Body almost unicolorous brown with an admixture of yellow in certain areas 5

5. Anteromarginal vestigial 6

   — Antermarginals short (10–20µm) 7

   — Anteromarginals long (> 25µm) 9

6. Antennal segments I & II brown, III – VIII golden yellow, III – VII pedicellate. Epimerals 150 µ long. Forewings with 17-20 double fringes

   ..... *nagaensis* Muraleedharan, 1982

7. Antennal segments IV–VI more rounded & pedicellate. S2 setae of tergum IX short but more than half as long as S1 & S3 …….. 8

   - Antennal segments IV–VI elongate not pedicellate. S2 setae very short, less than half the length of S1 & S3 ..... *parvus* Ananthakrishnan, 1976

8. Femora and tibia brown, apices yellow, all tarsi yellow. Body uniformly brown. Mesopraesternum more parallel sided .....*mimicus* (Ananthakrishnan, 1969)

   — Femora brown with apices yellowish; fore tibia yellow, mid and hind tibia pale brown with apices yellowish, tarsi largely yellow.

Mesopraestrnum boat shaped ..... *dentifer* (Priesner, 1935)

9. Mesopraesternum without median crest.....10

—Mesopraesternum with a median crest..... 13

10. Postocular short (<45µm) 11

— Postocular long (>45µm) 12

11. Anteroangulars and anteromarginals 20-25µm. S2 of tergite IX: 65, S1& S3 122-130µm long. Forefemora, mid and hind tibiae brown, mid and hind femora yellow at apex, fore tibiae yellowish brown, tarsi .....*dissimilis* Ananthakrishnan, 1976

— Anteroangulars and anteromarginals 30-40µm. S2 of tergite IX: 102-112, S1 & S3 140-155µm long. All femora and tibiae brown, tarsi yellow ..... *coorgensis* Ananthakrishnan, 1976

12. Postangulars short (60µm), S1, S2, S3 of tergiteIX respectively144-160, 64-80, 144-175µm. All legs uniformly yellow suffused with brown. Fore-wings with 9-10 double fringes .....*gallarum*Ananthakrishnan, 1967

- Postangular long (96µm), S1, S2, S3 of tergite IX respectively 172, 148, 256µ. All femora, mid and hind tibiae brown with golden yellow tinge, fore tibiae goldenyellow. Fore wings with 16-18 double fringes ..... *erraticus* Muraleedharan & Sen, 1981

13. Mouth cone broadly rounded.Antennal segments 1, 2, 7, 8 brown, 3-5 yellow, 6 basal yellow, apex brown. Fore wings with 12 - 14 double fringes. Fore tibia & all tarsi yellow, all femora & mid & hind tibia brown. Setae of tergite IX -S2: 72-85, S1 & S3: 91 - 106 µm ..... *davidi* Ananthakrishnan, 1976

— Mouth cone broad at base but slightly pointed at apex. Antennal segments 1, 2, 6 - 8 brown, 4 & 5 base yellow and proximal 2/3 brownish, 3 more yellow. Fore wings with 8-10 double fringes. All femora & tibiae yellowish brown,all tarsi yellow. S1, S2 & S3 of tergite IX respectively120-128, 64-72, 160-184 µm..... *nelliampathiensis* Varatharajan & Chochong, 2000

14. Post angular long (>40µm). Forewings with 6-7 doublefringes. Fore femora yellow, brown at base, mid and hind femora brown, distal tip yellow, fore tibia yellow, mid & hind tibiae and all tarsi brown..... *cacharensis* Muraleedhran & Sen, 1978

- Post angular short (<40µm). Fore wings with 12-15 double fringes. All femora brown, fore tibiae yellow, mid and hind tibiae brown with yellow apex..... *maoensis* Neelamani & Prasad, 1990

In all the 13 species listed in the table -1a, the length of S2 setae of abdominal segment IX was shorter than S1 and S3, whereas in the following three species such as *C. cacharensis, C. dantahastha* and *C. maoensis*, S1 and S2 are almost sub-equal and S3 is longer than S1 & S2. Absence of fore-tarsal tooth and equal length of S1 and S2 setae of tergite-IX in *C. cacharensis* resulted in naming it as *Inermothrips cacharensis*, i.e., *Inermothrips* as a sub genus of *Crotonothrips* (Muraleedhran & Sen, 1978). Since the above three species exhibit characters contrary to the definition of *Crotonothrips*, it is possible that they may come under the group of the genus *Liothrips.* As the data being not fully available for *C. dantahastha*, the key to identify this species is not provided here. However, an in-depth study is required on *C. cacharensis, C. dantahastha* and *C. maoensis* to consider them under any other genera, but as of now, the above three species are retained in the genus *Crotonothrips* (Thripswiki, accessed on 02.06.16).

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New evidence of pseudo scorpion *Ellingsenius indicus* Chamberlin as predator of Indian honey bee *Apis cerana* F.

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ABSTRACT: Traditional wall hives in the two villages of Kullu district, Himachal Pradesh namely Bhindi and Daula, recorded a heavy mortality of Indian honey bees (*Apis cerana*) due to the attack of an arachnid predator identified as a pseudo scorpion *Ellingsenius indicus*. It was observed that the pseudo scorpions did not venture the comb full of bees but attacked only those bees which were either moving in isolation or in groups of 1-3 or those coming and going to the hive entrance for foraging. There was a complete loss of bees in three conies whereas in other two colonies more than 70% of worker mortality was noticed. The observations recorded from the wall hives as well as from the laboratory experiments, revealed that generally 1-3 pseudo scorpions (*E. indicus*) caught hold of the single bee preferably from its legs and sometimes from its wings and did not leave the bee from their grip so long it was not dead.

KEY WORDS: Indian honey bee, wall hives, *Apis cerana,* pseudo scorpion, predator, *Ellingsenius indicus*

The presence of honey, beeswax and salubrious environmental milieu inside a bee hive/nest, invite and entice number of insects, mites and other visitors. Some feed on honey, others on wax, some simply refuse inside the hive to enjoy warmth and still others feed on bees. The pseudo scorpion *Ellingsenius indicus* Chamberlin associated with honey bees has been observed by many beekeepers and researchers and it was reported that these individuals were melittophilic and it was believed that they did not cause harm to bees but use them phoretically for dispersal (Murthy and Venkataraman, 1985). Donovon and Paul (2006) have reported *E. indicus* eating arthropods enemies of honey bees including varroa mite (*Varroa jacobsoni*) in the colonies of Indian honey bees (*Apis cerana*) and reported that honey bees were not attacked by *E. indicus*. Later on Thapa *et al*. (2013) reported that pseudo scorpions did not prey on mites and lesser wax moth larvae but on the dead honey bees, bee larvae and live psocids. Investigations were carried out on the basis of the information provided by the local beekeepers of Kullu district of Himachal Pradesh regarding the mass scale mortality of bees and perishing of their traditional wall hive colonies of Indian honey bee (*A. cerana*).

During April-May, 2014 farmers from Bhindi (from 31^0 50'-53" north latitude and 77^0 08'- 55" east longitude, situated at an elevation of 1362 metres above mean sea level) and Daula (from 31^0 50'-

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71'' north latitude and 77^0 08'-95'' east longitude situated at an elevation of 1324 metres above mean sea level) villages in Kullu district of Himachal Pradesh reported heavy mortality of Indian honey bees, *Apis cerana* in their traditional wall hives due to the attack of an arachnid. The villages were visited and the photographs of the arachnid attacking and killing the bees were taken and the thorough observations on the mode of predation of *A. cerana* bees by the arachnids were recorded. The live as well as dead specimens of the arachnid were brought to the laboratory.

The specimens were identified as pseudo scopions belonging to the order, Chelonethi; superfamily, Pseudoscorpionidea; family, Cheliferidae; species *Ellingsenius indicus*. The full grown pseudo scorpions were 7.9 ± 0.03 cm long with dark brown colour whereas the nymphs were pale white in colour. Both adults and nymphs were found roaming freely in the cells of the combs where bee activity was quite less or sometimes negligible and were also found in the cracks and crevices near the base of the wall hives (Photo 1).

The live specimens of E. *indicus* were brought to the laboratory and five live *A. cerana* bees along with five healthy full grown larvae (for keeping the pseudo scorpions alive after they killed the living bees) were put in each of the three cages covered with muslin cloth and four pseudo scorpions were released in each of the three cages and the observations were recorded on the predation of the bees by the pseudo scorpions for a period of 1 hour daily for 7 days. After taking observations each day, the dead bees were removed along with the larvae and fresh live bees and bee larvae were put in the cages. The dead pseudo scorpions were also replaced by the live ones from time to time to maintain their number 4 everyday in each cage.

In the wall hives of Bhindi and Daula villages of of Kullu district, it was observed that pseudo scorpion did not venture the comb having flurry of bee activity but attacked only combs where activity was less and at those places on the comb (particularly on the lower sides of the combs) where bees were either moving in isolation or in groups of 2-3 or those coming and going to the hive entrance for foraging. The attack of pseudo scorpions was noticed in five colonies in wall hives in two villages (Bhindi = 2, and Daula = 3) and there was a complete loss of bees in three conies whereas in other two colonies more than 70% of worker mortality due to pseudo scorpions was noticed and these two colonies got absconded. The observations recorded from the wall hives and from the laboratory experiments revealed that generally 1-3 pseudo scorpions (*E. indicus*) caught hold of the single bee preferably from its head or legs and sometimes from its wings and did not leave the bee from their grip so long it was not dead (Photos 2-3) After injecting saliva into the victim, they feed on liquefied contents. Having sucked the haemolymph of its prey, the pseudo scorpions shift their focus to the other bee.

The data recorded for 7 days showed that the time taken for 12 pseudo scorpions to kill fifteen bees per day varied between 29.33 to 33.33 minutes and the average time taken for a single predator to kill its prey (bees) varied between 2.44 to 2.67 minutes. There was a huge reduction in the size of bee killed by the pseudo scorpions whereas the latter inflated in their size. The data on the longevity of the pseudo scorpions revealed that during second, third, fourth, fifth and sixth day, the mortality was found to be 41.66, 66.66, 83.33, 91.66 and 100 per cent respectively. The higher mortality of the predators (pseudo scorpions) was also recorded because of the counter attack of the honey bees to fend off themselves and ensuing melee between them.

The present investigations from the farmer's locality as well as from the laboratory clearly indicated that pseudo scorpions predated upon live *A. cerana* bees and preferred live to dead bee larvae. However in the absence of the live adult bee, they preyed on the live bee larvae. of Thapa *et al.* (2013) who reported that pseudo scorpions associated with *A. cerana* prey on dead honey bees, bee larvae and psocids. But in the present studies it was found that under laboratory conditions pseudo scorpions did not prefer to take dead bees as well as larvae

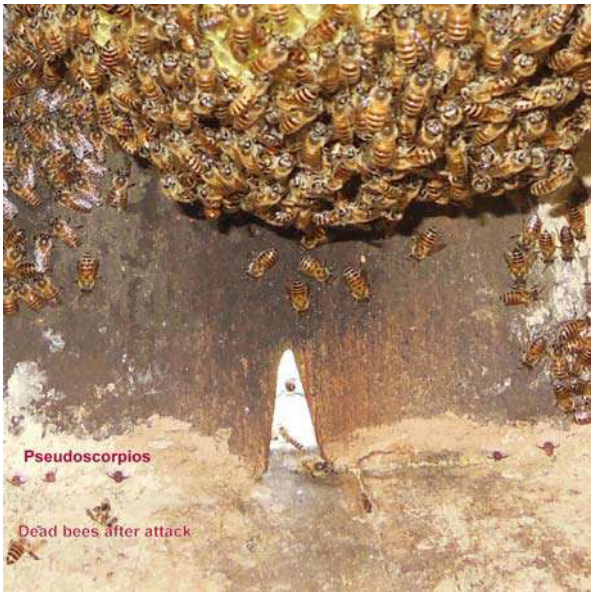

Fig 1

Fig 3

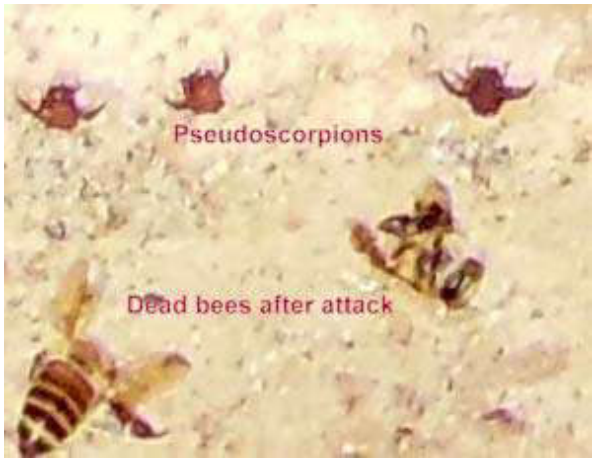

Fig 2

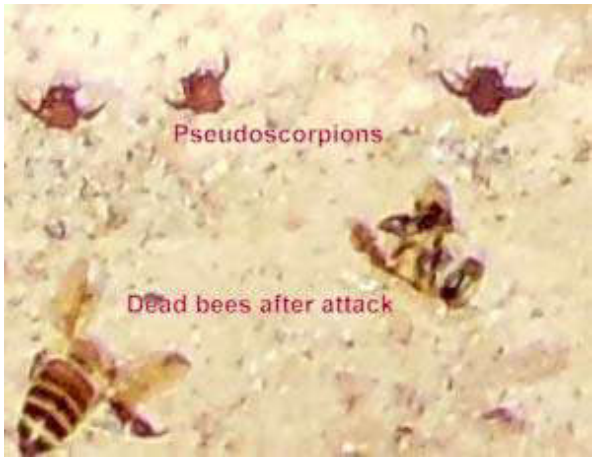

Fig 2-1

as their food so long they had access to live ones. Earlier Subbiah *et al*. (1957) had reported that the extent of harm done by *E. indicus* to bees was not exactly known but it was certainly a hindrance for the foraging activities of bees. Randy (2003) also reported that *E. indicus* sometimes feed on injured honey bees but was usually more interested in feeding on other insects like wax moth larvae and honey bee mites. However the present findings are contrary to the findings of Murthy and Venkataramanan (1985) and Semmer *et al*. (2014) who reported that *E. indicus* associated with *A. cerana* bees do not harm bees but use them phoretically for dispersal. Donovan and Paul (2006) have reported pseudo scorpions *E. indicus* eating arthropod enemies of honey bees including varroa mite (*Varroa destructor*) and it was also reported by them that the *E. indicus* did not attack the honey bees. However, Gonzalez *et al*. (2007) have reported that the role of pseudo scorpions within bee nests is still poorly known and the most records of pseudo scorpion-bee relationship are sporadic observations and are sparsely reported in the literature. Present observations clearly showed that *E. indicus* is a predator of Indian honey bees and is a potential danger for these indigenous honey bees in future.

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First report of the invasive rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) from the Old World

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ABSTRACT: Occurrence of the Rugose spiraling whitefly (RSW), *Aleurodicus rugioperculatus* Martin is reported for the first time from the Old World. *Aleurodicus rugioperculatus* is compared with *A. dispersus* Russell, the only con-generic species known from India. *Encarsia guadeloupae* Viggiani (Hymenoptera: Aphelinidae) parasitises *A. rugioperculatus*. Host range of the RSW is discussed and ten new host records are provided.

KEY WORDS: Rugose spiraling whitefly, *Aleurodicus rugioperculatus, Encarsia guadeloupae*, host plants, India

The whitefly genus *Aleurodicus* Douglas encompasses 35 species, of which only the spiralling whitefly *Aleurodicus dispersus* Russel was so far known to occur in India (Martin, 2008). The Rugose Spiraling Whitefly (RSW) (*Aleurodicus rugioperculatus*) was described by Martin from Belize in Central America in 2004 based on puparia collected under the leaves of Coconut. It invaded Florida in the United States in 2009 and Guatemala (Stocks, 2012) and since then its range expanded considerably within the United States (Antonio *et al.,* 2016). The RSW is highly polyphagous with 118 hosts belonging to 43 plant families including economically important crops in the United States (Antonio *et al.,* 2016).

A severe outbreak of the RSW, so far confined to the Americas, was noticed on Coconut palms, Mango and Guava at Changanassery, Kottayam District, Kerala in India following accidental introduction (Fig.3A to 3D). The females lay wax covered eggs in a spiral fashion usually on the abaxial surface of leaves. Prolific feeding by the nymphs and adults on coconut trees resulted in copious honeydew that covered the undergrowth of plants which in turn became black due to the development of sooty mould.

Five field surveys were carried out in Kottayam district, Kerala covering 15 locations based on distress calls received from farmers growing rice and coconut. Pieces of coconut leaves bearing puparia were collected in 70% ethyl alcohol. Permanent microscopic slides were prepared following Martin, 2004. Parasitised puparia were kept in insect breeding dishes for the emergence of parasitoids which were then transferred to 95% ethyl alcohol. Microphotographs were taken using

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Leica MC170 HD digital camera mounted on Leica DM2000 LED compound microscope and a Canon EOS 1100D digital camera mounted on Leica M205C stereo microscope. The images were stacked using CombineZP and edited using Adobe Photoshop.

The slides of *Aleurodicus rugioperculatus* will be deposited in the Natural History Museum, London and the Travancore Insect Collection, Department of Agricultural Entomology, Kerala Agricultural University, Vellayani.

Diagnosis: The Rugose spiraling whitefly adults (Figs. 4 & 5) are much larger than the common silver leaf whitefly, *Bemisia tabaci* G. (Fig. 6). Both *A. rugioperculatus* and *A. dispersus* possess four large compound pores on the abdominal segments III to VI. However, they can be easily differentiated based on the following characters of the puparia given in Table 1.

Host plants of *Aleurodicus rugioperculatus*: Stocks and Hodges (2012) reported about 95 host plants of *A. rugioperculatus* in Florida, USA. Further, Antonio *et al.* 2016 reported a broader host range of 118 species in 43 families. In the present study, a total of 17 plant species in 11 families were recorded as hosts of *A. rugioperculatus* (Table 2), of which 10 are new.

Table 1. Distinguishing puparial characters of Rugose spiralling whitefly and spiralling whitefly

No.	Puparial character	*Aleurodicus rugioperculatus*	*Aleurodicus dispersus*
1.	Cuticle on Dorsum	Reticulated (Fig. 1A, 1B)	Smooth (Fig. 2A)
2.	Compound pores on abdominal segments VII & VIII	Present (Fig. 1A, 1C)	Absent (Fig. 2A, 2C)
3.	Corrugations / rugosity on the surface of operculum	Present (Fig. 1D)	Absent (Fig. 2A, 2C)
4.	Shape of the apex of lingula	Acute (Fig. 1D)	Oval (Fig. 2D)

Table 2. Host plants of *A. rugioperculatus* in Kerala

Sl. No.	Scientific Name	Family	Common Name
1.	*Cocos nucifera* L.	Arecaceae	Coconut
2.	*Musa* sp.	Musaceae	Banana
3.	**Artocarpus* hirsutus Lam.	Moraceae	Wild Jackfruit
4.	*Artocarpus heterophyllus* Lam.	Moraceae	Jackfruit
5.	**Ficus exasperata* Vahl..	Moraceae	Brahma's Banyan
6.	*Mangifera indica* L.	Anacardiaceae	Mango
7.	*Psidium guajava* L.	Myrtaceae	Guava
8.	**Acacia mangium* Willd.	Fabaceae	Brown salwood
9.	**Garcinia gummi-gutta* (L.)	Clusiaceae	Malabar tamarind
10.	*Thespesia populnea* (L.)	Malvaceae	Portia tree
11.	**Sida acuta* Burm. f.	Malvaceae	Wire weed
12.	*Terminalia catappa* L.	Combretaceae	Indian Almond
13.	**Combretum indicum* (L.)	Combretaceae	Rangoon creeper
14.	**Allamanda cathartaca* L.	Apocynaceae	Golden trumpet
15.	**Nerium oleander* L.	Apocynaceae	Oleander
16.	**Codiaeum verigatum* (L.)	Euphorbiaceae	Garden croton
17.	**Euphorbia milii* Des Moul.	Euphorbiaceae	Crown of thorns

* New Host Record

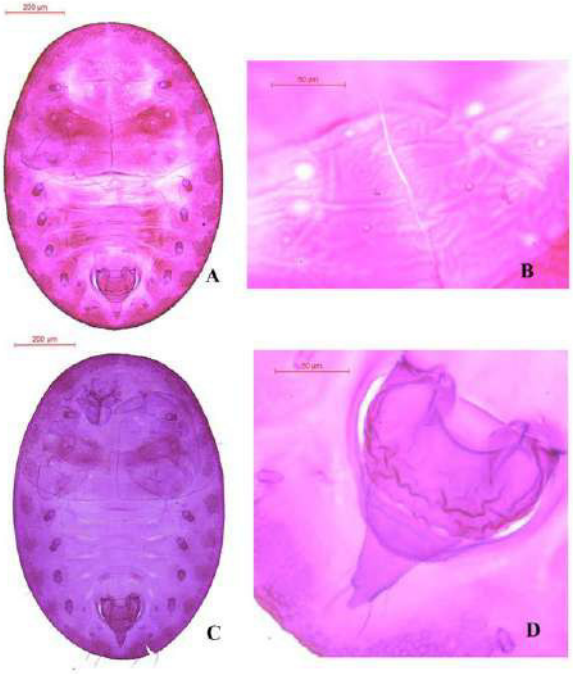

Fig.1. ***Aleurodicus rugioperculatus*** Martin, puparium, **A.** Dorsum **B.** Reticulate sculpture on cephalothorax, **C.** Venter, **D.** Operculum and lingula

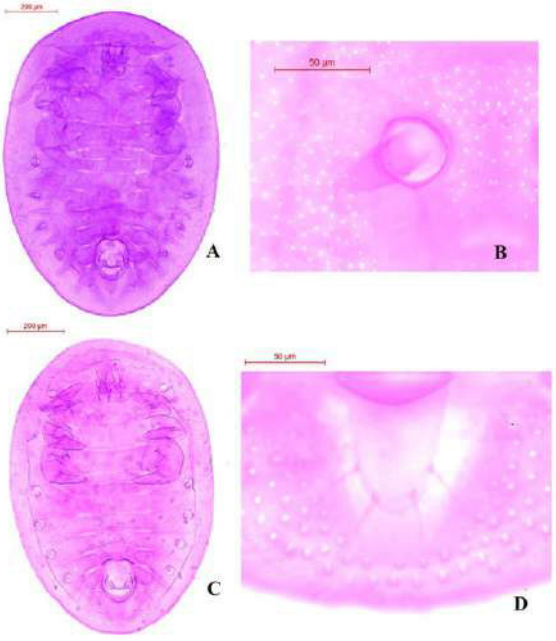

Fig.2. ***Aleurodicus dispersus*** Russell. **A.** Dorsum **B.** Compound Pore, **C.** Ventral, **D.** Lingula

Aleurodicus rugioperculatus being a recent introduction, is still in the process of adapting and establishing on various native plants in India. Hence the species was observed only on lesser number of host plants in India compared to those in North America. The host range is likely to expand as the species becomes more established and spread to newer areas in India.

Natural Enemies: *Encarsia guadeloupae* Viggiani (Hymenoptera: Aphelinidae), a well known parasitoid of *A. dispersus* (Ramani *et al.* 2002; Evans, 2007), was found to parasitise *A. rugioperculatus*. This has already been reported on *A. rugioperculatus* from Florida (Kumar *et al.,* 2013; Taravati *et al.,* 2013) and appears to be a potential biocontrol agent against RSW as 50 to 60% natural parasitisation of the pupae was observed (Figs. 7, 8A to 8G).

Mode of entry of RSW into India is unknown. However, it is likely that the pest gained entry into the country through trade in ornamental plants. Having been introduced, it may be impossible to contain spread and establishment of the pest in India. Hence sustainable pest management practices should immediately be initiated.

Fig.3. Host plants heavily infested with rugose spiraling whitefly, ***Aleurodicus rugioperculatus*** Martin. **A.** Coconut Leaf, **B.** Coconut leaf petiole, **C.** Mango leaves, **D.** Guava leaf.

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Fig.4 Fig.5 Fig.6 Fig.7

Fig.4. ***A. rugioperculatus*** adult. Female, **Fig.5**. ***A. rugioperculatus*** adult. Male, **Fig.6**. ***Bemisia tabaci*** adult, **Fig.7**. Parasitoid exit hole on ***A. rugioperculatus*** puparium.

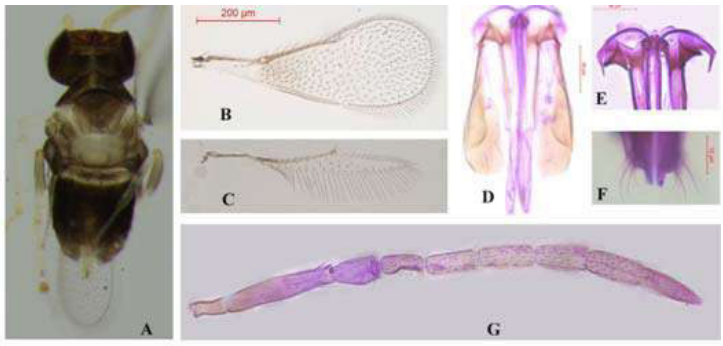

Fig.8. ***Encarsia guadeloupae*** Viggiani (Hymenoptera: Aphelinidae) **A.** Adult, **B.** Fore wing, **C.** Hind wing, **D.** Ovipositor, **E, F.** Ovipositor (in part), **G.** Antennae.

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